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SPECIATION DUE TO HYBRID NECROSIS IN PLANT–PATHOGEN MODELS

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We develop a model for speciation due to postzygotic incompatibility generated by autoimmune reactions. The model is based on frequency-dependent interactions between host plants and their pathogens, which can generate disruptive selection and give rise to speciation if distant phenotypes become reproductively isolated. Based on recent experimental evidence from *Arabidopsis*, we assume that at the molecular level, incompatibility between host strains is caused by epistatic interactions between two proteins in the plant immune system—the guard and the guardee. Within each plant strain, immune reactions occur when the guardee protein is modified by a pathogen effector, and the guard subsequently binds to the guardee, thus precipitating an immune response. When guard and guardee proteins come from phenotypically distant parents, a hybrid's immune system can be triggered by erroneous interactions between these proteins even in the absence of pathogen attack, leading to severe autoimmune reactions in hybrids. This generates a Dobzhnasky–Muller incompatibility due to immune reactions. Our model shows how phenotypic variation generated by frequency-dependent host–pathogen interactions can lead to such postzygotic incompatibilities between extremal types, and hence to speciation.

KEY WORDS: **Autoimmune reaction, evolution of diversity, hybrid necrosis, hybrids, immune system, speciation.**

Understanding the origins of diversity is a central theme in evolutionary biology. In general, evolutionary diversification can be described as a temporal modification of phenotype distributions (Coyne and Orr 2004; Doebeli et al. 2007). A single species typically corresponds to a unimodal phenotype distribution, whereas this distribution becomes bimodal (or multimodal) once diversification has occurred (Doebeli et al. 2007). Thus, speciation can be described as a splitting of the ancestral unimodal phenotype distribution into two or more descendant peaks, with each peak corresponding to an emerging species. Traditionally, diversification and speciation are thought to occur when different phenotypes are selectively favored in different and isolated geographical regions, so that an initially uniform ancestral population that is geographically dispersed would develop different phenotypic modes corresponding to the phenotypes favored in different locations. More recently, processes of adaptive speciation, which unfold in the absence of geographical isolation and during which phenotype distributions become multimodal due to ecological interactions such as competition for resources of predation, have received considerable attention (Dieckmann et al. 2004). In sexual populations, adaptive speciation requires that the different clusters in the phenotype distribution, corresponding to the newly emerging species, are separated by barriers to gene flow. That is, to prevent mixing between the emerging species, successful reproduction should occur predominantly within each emerging phenotypic cluster, and not between individuals with distinctly different phenotypes. The type of reproductive isolation that has been considered in most theoretical models of adaptive speciation is isolation due to assortative mating, i.e., prezygotic isolation (e.g. Dieckmann and Doebeli 1999; Dieckmann et al. 2004; Doebeli 2005; Bürger and Schneider 2006; Bürger et al. 2006; Pennings et al. 2008). In this article, we investigate the potential role of postzygotic isolation for adaptive speciation by considering models in which postzygotic isolation is caused by autoimmune responses leading to Dobzhansky–Muller incompatibilities (Gavrilets 2004).

Recently, Eizaguirre et al. (2009) have pointed out that the immune system may play an important role in processes of diversification. These authors primarily considered the role of the vertebrate immune system for prezygotic isolation (so that mating partners would be chosen based on immune system characteristics), but they also mentioned that the immune system may be important for postzygotic isolation due to a weakened immune response in hybrids. Here, we consider a different type of hybrid disadvantage due to malfunctioning of the immune systems, which is based on observations made in plant systems. In plants, a well-known example of postzygotic isolation is hybrid necrosis, defined as a set of highly deleterious and often lethal phenotypic characteristics (Bomblies and Weigel 2007). In hybrid necrosis, a mixture of genes from different strains becomes deleterious even though the contributing genes were harmless, or even beneficial, in the parents. Recent experimental evidence suggests that hybrid necrosis in plants can be caused by an epistatic interaction of loci controlling the immune response to attack by pathogens (Bomblies et al. 2007). In this form of hybrid necrosis, inviability is caused by inappropriate activation of the plant immune system in the absence of pathogens. Among several mechanisms of pathogen recognition by a plant host cell, interactions between two different types of host proteins, "guard" and "guardee" proteins, are thought to play a key role (Jones and Dangl 2006). When a pathogen attacks a host cell, it often injects effector proteins that manipulate target proteins in the host cell and thereby contribute to the success of the pathogen. These host targets (the guardees) are "guarded" by other host proteins (the guard) that monitor the guardee's molecular structure. When a pathogen effector induces changes in the molecular structure of the guardee (creating "pathogen-induced modified-self"), these changes are recognized by the guard proteins, which then activate the immune response (Jones and Dangl 2006). Thus, on the one hand successful pathogen attack requires fine-tuning of the effector to the guardee, so that a pathogen with a given effector repertoire can only successfully attack a certain range of host cells (i.e., those with the "right" types of guardees). On the other hand, efficient immune response requires fine-tuning of the guardee and the guard, so that the guard selectively recognizes only those guardees that have been modified by a pathogen. However, if mating between different host strains leads to hybrids in which guard and guardees come from lineages with different evolutionary paths, the guard might recognize the guardee as modified even in the absence of pathogen attack, which could lead to immune response and subsequent necrosis of the hybrid even in the absence of any pathogen. Experimental evidence of two-locus epistatic interactions for hybrid necrosis, of the autoimmune nature of the deleterious phenotype, and of an increased disease resistance of hybrids rescued from necrosis all support the hypothesis that guard–guardee interactions are involved in hybrid necrosis in the well-studied model plant *Arabidopsis thaliana* (Bomblies et al. 2007). A large number of hybrid necrosis cases sharing phenotypic similarities with the *Arabidopsis* cases indicate that this may be a common mechanism operating in a wide range of plant species.

Due to the potentially high selection pressures exerted by pathogen attack, genes controlling immune responses are generally thought to be fast evolving. This can, in turn, generate strong selection pressures on pathogens, which can lead to coevolution. In addition, such host–pathogen, or more generally, predator–prey interactions, are often frequency-dependent, and it is known that this frequency dependence can generate disruptive selection in the host (Dercole et al. 2003; Doebeli and Dieckmann 2000). Here we present a mathematical model of adaptive speciation in a host plant in which diversification is driven by host–pathogen interactions, and postzygotic reproductive isolation is caused by hybrid necrosis. The barriers to gene flow between emerging phenotypes are generated by detrimental autoimmune reactions in individuals containing pathogen-resistance genes from different clusters. Specifically, reproductive postzygotic isolation is due to genetic incompatibility between guard and guardee proteins of phenotypically distant plant strains. In the following, we develop a simple representation of the evolution of the guard and guardee proteins driven by selection for escaping recognition of the guardee by pathogen effectors. As we will show, the frequency-dependent selection imposed by the pathogen leads to the emergence of distinct strains that are reproductively isolated due to genetic incompatibility in the immune response. The emerging host strains correspond to two distinct paths of coordinated evolution of guard and guardee proteins. Mating between these strains can result either in hybrids that are very disease prone due to a defective immune system, or in individuals that show hybrid necrosis due to autoimmune reactions.

Model Description

PHENOTYPE SPACE

Our aim is to describe a minimal model with which to investigate the dynamics of hybrid necrosis, and several experimental observations from Bomblies et al. (2007) are essential for the definition of our model. First, it has been uncovered that the autoimmune reaction plays an essential role in the lethality of the hybrid phenotype, and that the surviving hybrids exhibit increased pathogen resistance. Second, it was shown that epistatic interactions between two loci are both a necessary and a sufficient condition for hybrid necrosis. And finally, it was observed that an increase in a habitat temperature from 16°C to 23°C rescues the hybrid. All these phenomena strongly indicate the possibility that hybrid necrosis is caused by an erroneous binding between guard and guardee proteins coming from different parents. In normal plants, such a binding only occurs if the guarded protein is modified by

the pathogen effector. However, in a hybrid, such a binding could happen in the absence of pathogen effectors because the guard and guardee proteins evolved independently and could thus have acquired a propensity for binding, i.e., a higher binding energy, without any additional alterations through pathogen effectors. An increase in the ambient temperature weakens any binding, thus decreasing a chance to provoke the unwarranted immune response in a hybrid, exactly as observed in Bomblies et al. (2007). An estimate showing that the increase in ambient temperature applied in the experiments can indeed cause a noticeable shift in binding equilibrium of guard and guardee proteins is presented in the Appendix . For our model, we envisage biochemical binding between guard and guardee proteins to be the mechanism for both immune and autoimmune responses.

We assume that the evolution of guardee and guard protein is described by two phenotypic coordinates, g and r. Each host plant individual is represented by a point in this two-dimensional phenotype space, with the g-coordinate describing the state and genetic makeup of the individual's guardee protein and its important interaction partners, and the r-coordinate summarizes the properties of the individual's guard protein. Essentially, the r and g coordinates should be thought of as projections of some high-dimensional vectors that characterize the functioning of guard and guardee proteins and their ability to bind to each other and induce an immune response.

Mutations in guard and guardee genes cause the corresponding point to shift in (r, g) space. The density of plant individuals with a particular form of guard and guardee proteins is described by a density distribution function $h(r, g)$. An almost homogeneous population with little genetic variation in immune proteins is described by a density distribution with a single narrow peak, whereas a population consisting of several strains with different guard and guardee proteins is described by a ultimodal density distribution.

In our framework, the pathogen is characterized by a single coordinate, e, which describes the genetic makeup of the pathogen's effector proteins. Similar to the definition of r and g, the coordinate e should be thought of as a one-dimensional projection of some multidimensional vector, so that e conveys only the characteristics related to the ability of the effector to induce modifications of the plant that are favorable for the pathogen. The effector proteins are assumed to interact with the guardee protein of a host plant such that the effectiveness of pathogen attack is determined by some matching with the plants g coordinate, as in many traditional predator–prey models (e.g., Doebeli and Dieckmann 2000). After appropriate rescaling of the pathogen trait, we can then assume that the pathogen attack is most effective when $e = g$ and becomes less effective when distances $|e - g|$ between the pathogen coordinate e and the host's guardee coordinate g become larger. Thus, it is in the interest of the host to have a guardee phenotype that is detuned from the specialization of the most common pathogen, and this is the mechanism that generates frequency-dependent selection.

In the plant host, the relative values of the r and g coordinates determine the immune reaction of the plant to a pathogen effector, as well as autoimmune reactions. We assume that there is a trade-off between mounting an efficient immune response in the presence of pathogen effectors, and being prone to deleterious autoimmune reactions in the absence of pathogens. Specifically, we assume that when $g \gg r$, the probability that the guard binds to the guardee protein and triggers an immune response is small, independent of whether pathogen effectors are present or not. In this case, the plant immune system is less sensitive, making the plant more susceptible to pathogen attack, but less prone to autoimmune reactions. Conversely, when $r \gg g$, the guard protein has a high propensity to bind to the guardee. In this case, the plant immune system is more sensitive, making the plant less susceptible to pathogen attack, but more prone to autoimmune reactions. This parameterization of phenotype space is in accord with the observation made in Bomblies et al. (2007) that necrotic hybrids, being rescued by elevated ambient temperature, are very effective in suppressing pathogen attacks.

Quantitatively, we assume that the probability for an (r, g)-plant to die from parasitic infection once attacked by a pathogen is proportional to $\exp[(g - r)/\sigma_I]$, whereas the probability to die from autoimmune reactions is proportional to $\exp[(r - g)/\sigma_{AI}]$. Here, σ_I and σ_{AI} are system parameters that reflect the plant's sensitivity to the immune response. As a consequence of this trade-off, for $\sigma_I \approx \sigma_{AI}$, plants tend to survive best if their phenotypes satisfy $r \approx g$.

PLANT AND PATHOGEN EVOLUTION

To describe the coevolution of the plant and pathogen populations, we use a predator–prey-style model for the dynamics of phenotype distributions in both the host plant and the pathogen, with specific terms that describe the plant–pathogen conflict and autoimmune reactions. Mathematically, the model is a system of two integro–differential equations that gives the temporal evolution of the host density distribution $h(r, g)$ and the pathogen density distribution $p(e)$. To simplify the notation, we do not show explicit time dependence of the density distributions in the following. The equations determining the rate of change in the distributions $h(r, g)$ and $p(e)$ consist of birth and death terms, describing the increase and decrease in plant and pathogen population densities due to the various biological components and interactions, as follows.

- For a given plant phenotype (r, g), the rate of attack from the pathogen, and hence the probability of infection, is proportional to a weighted sum over all pathogen phenotypes, with the weights reflecting how well a pathogen phenotype e can

attack a plant with guardee phenotype g. The weight function, or "attack kernel," is assumed to be of Gaussian form and given by

$$G_a(e-g) = \exp\left[-\frac{(e-g)^2}{2\sigma_a^2}\right], \quad (1)$$

reflecting the fact that infection is easiest if the pathogen phenotype is very similar to the guardee phenotype (with the sensitivity of attack efficiency to deviation in $|e-g|$ from 0 determined by the characteristic width σ_a). Accordingly, for a given plant phenotype (r, g), the probability of being attacked is proportional to

$$\int p(e)G_a(e-g)\,de. \quad (2)$$

Once attacked, the probability that the plant individual dies is determined by the immune response (as described above) and is proportional to $\exp[(g-r)/\sigma_I]$, reflecting how well the guard protein recognizes the changes in the guardee protein induced by the pathogen attack. Overall, this leads to a total death rate of plants with phenotypes (r, g) due to pathogen attack given by

$$-\delta_I h(r,g)\exp\left(\frac{g-r}{\sigma_I}\right)\int p(e)G_a(e-g)\,de. \quad (3)$$

Here δ_I is a constant of proportionality, and the minus sign indicates that this is a death term.

- In the plant population, death also occurs due to autoimmune reactions, whose magnitude in plants of phenotype (r, g) is proportional to $\exp[(r-g)/\sigma_{AI}]$. The resulting death rate is given by

$$-\delta_I h(r,g)\exp\left(\frac{r-g}{\sigma_{AI}}\right). \quad (4)$$

For simplicity, we assume that the rate coefficient δ_I for the autoimmune and pathogen-induced death terms is the same.

- To complete the death terms for the plant, we assume that density-dependent competition in the absence of the pathogen results in a logistic death term of the form

$$-\delta_C\frac{h(r,g)H}{K(r,g)}, \quad (5)$$

here $H(t)$ is the total density of the plant population, i.e.,

$$H = \int_{r,g} h(r,g)\,dr\,dg. \quad (6)$$

The parameter δ_C in equation (5) is a rate coefficient for the plant death rate due to competition. The function $K(r, g)$ is the carrying capacity function, which we assume to be of the form

$$K(r,g) = \exp\left[-\frac{(r-r_0)^2}{2\sigma_r^2}\right]\exp\left[-\frac{(g-g_0)^2}{2\sigma_g^2}\right]. \quad (7)$$

This reflects the assumption that there are optimal values r_0 and g_0 for guard and guardee traits, which are determined by their costs and benefits to the plant and which are unrelated to their immune function. This means that in the absence of a pathogen attack and immune response, the plant traits would evolve to their optimal values r_0 and g_0 (and the plant distribution would converge to a narrow unimodal distribution centered at (r_0, g_0)). In this way, the carrying capacity function K provides a component of stabilizing selection that is independent of host–pathogen interactions.

In general, it is not clear what the position of (r_0, g_0) would be in phenotype space. In particular, we do not know what cost stabilizing selection would impose on a well-tuned immune response, for which $r \approx g$. Therefore, we assume in the following that only the trait associated with the guardee (g) protein affects the carrying capacity. As this is also the trait that affects the host–pathogen interaction, this corresponds to commonly made assumptions (e.g., Doebeli and Dieckmann 2000). Mathematically, the fact that r-trait does not affect the carrying capacity corresponds to the assumption that the width of the carrying capacity in the r-direction is very large, $\sigma_r \to \infty$.

- To derive the birth term for the plant population, we need to incorporate sexual reproduction, and for simplicity we assume that individuals are haploid and have two loci with continuously varying alleles encoding the two phenotypes r and g. An offspring with phenotype (r', g') either inherits the two alleles r' and g' from different parents, or from the same parent. In the first case, the offspring comes from a mating between (r', g'') and (r'', g') (or vice versa) with all possible g'' and r''. Such matings occur with probability proportional to $\frac{h(r',g'')h(r'',g')}{2H}$, where H is the total plant density as before. In the second case, the probability that such an offspring is produced is simply proportional to $h(r', g')$. Adding the two cases together, the total probability that an (r', g') offspring is the result of mating is thus

$$\int\frac{h(r',g'')h(r'',g')}{2H}dr''dg'' + \frac{h(r',g')}{2} \quad (8)$$

(the factor $1/2$ reflects ambiguity in assigning mother and father in any given mating pair). In addition, we assume that mutation in the plant traits is described by two normal mutation kernels

$$G_{M,r}(r-r') = \frac{1}{\sqrt{2\pi}\sigma_{M,r}}\exp\left[-\frac{(r-r')^2}{2\sigma_{M,r}^2}\right] \quad (9)$$

and

$$G_{M,g}(g-g') = \frac{1}{\sqrt{2\pi}\sigma_{M,g}} \exp\left[-\frac{(g-g')^2}{2\sigma_{M,g}^2}\right]. \tag{10}$$

Here $G_{M,r}(r-r')$ describes the probability that a mutation shifts the r'-allele to r, and similarly for $G_{M,g}(g-g')$. Thus, small mutations are more likely than large ones.

Overall, the rate at which offspring with phenotype (r, g) are produced is then given by

$$\beta \int\int G_{M,r}(r-r')G_{M,g}(g-g') \times \left[\int\int \frac{h(r', g'')h(r'', g')}{2H} dr''dg'' + \frac{h(r', g')}{2}\right] dr'dg', \tag{11}$$

where β is the birth rate.

- For the pathogen, birth is determined by successful infection. For pathogen type e', the probability that it successfully attacks and subsequently infects a host of type (r, g) is proportional to

$$h(r, g) \exp\left(\frac{g-r}{\sigma_I}\right) G_a(g-e'), \tag{12}$$

where G_a is the attack kernel given by equation (1). Therefore, the total probability per unit time for pathogen type e' to successfully infect any host plant is

$$\int\int h(r, g) \exp\left(\frac{g-r}{\sigma_I}\right) G_a(g-e')\, drdg. \tag{13}$$

For simplicity, we assume that reproduction is asexual in the pathogen, but as in the plant species we include mutation given by a normal function $G_{M,e}$ (with a width described by a parameter $\sigma_{M,e}$), so that the total rate of production of offspring of type e is

$$\alpha\delta_I \int G_{M,e}(e-e')p(e')\left[\int\int h(r, g) \exp\left(\frac{g-r}{\sigma_I}\right) \times G_a(g-e')\, drdg\right] de'. \tag{14}$$

Here the conversion coefficient α indicates how many new pathogens are produced from an infected host.

- Finally, as in many other predator–prey models, the death rate of the pathogen is assumed to be

$$-\delta_P p(e), \tag{15}$$

for some parameter δ_P describing the intrinsic per capita death rate of the pathogen. Note that we assume that this death rate is independent of the pathogen phenotype e.

Collecting all the birth and death terms into two equations describing the dynamics of the plant and pathogen density distributions now yields the following system of coupled first-order partial differential equations:

$$\frac{\partial h(r, g)}{\partial t} = \beta \int\int G_{M,r}(r-r')G_{M,g}(g-g') \times \left[\int\int \frac{h(r', g'')h(r'', g')}{2H} dr''dg'' + \frac{h(r', g')}{2}\right] dr'dg' - \delta_I h(r, g) \exp\left(\frac{g-r}{\sigma_I}\right) \int p(e)G_a(e-g)\, de - \delta_I h(r, g) \exp\left(\frac{r-g}{\sigma_I}\right) - \delta_C \frac{h(r, g)H}{K(r, g)}, \tag{16}$$

$$\frac{\partial p(e)}{\partial t} = \alpha\delta_I \int G_{M,e}(e-e')p(e')\left[\int\int h(r, g) \exp\left(\frac{g-r}{\sigma_I}\right) \times G_a(g-e')\, drdg\right] de' - \delta_P p(e). \tag{17}$$

These nonlinear equations have many parameters and in principle may exhibit a variety of dynamic regimes. Due to the apparent complexity of the dynamical system, we do not expect any analytical results to be feasible and instead investigated this system using numerical simulations. We are particularly interested in those regimes that lead to multimodal equilibrium distributions in the host plant.

Such dynamics are illustrated in Figure 1, occur for a substantial range of parameters and correspond to cases in which the host–pathogen interaction leads to plant speciation. Intuitively, the choice of parameters generating this scenario can be explained as follows: The width of the carrying capacity σ_g defines the phenotypic space scale of the model and is taken to be 1. The birth coefficient of the host, β, defines the time scale and is also taken to be 1. Finally, the competition death term defines the third scale, the amplitude of the population density, and is taken to be one as well. The mutation widths for the host $\sigma_{M,r}$, $\sigma_{M,g}$, and pathogen $\sigma_{M,e}$ define the minimal width of the emerging pattern and should be significantly smaller than the width of the host carrying capacity function, which defines the "playing field" where multimodality can develop. Also, to enable pattern formation due to host–pathogen interaction, the pathogen attack width should be small enough compared to the width of the carrying capacity, and our simulations indicate that being smaller than the width of the carrying capacity is generally small enough. Finally, the intensity of the autoimmune reaction and the susceptibility to the pathogen infection should grow sufficiently fast (i.e., faster than the decay in carrying capacity) as the phenotypic distance from the optimum, $r \approx g$, increases. Thus, the immune and autoimmune reaction widths σ_I and σ_{AI} must be noticeably less than the width of the carrying capacity. Other rates are set equal to unity except for the coefficients for the autoimmune and pathogen-induced death term, δ_I, which, to make this term more significant, is set equal to 5.

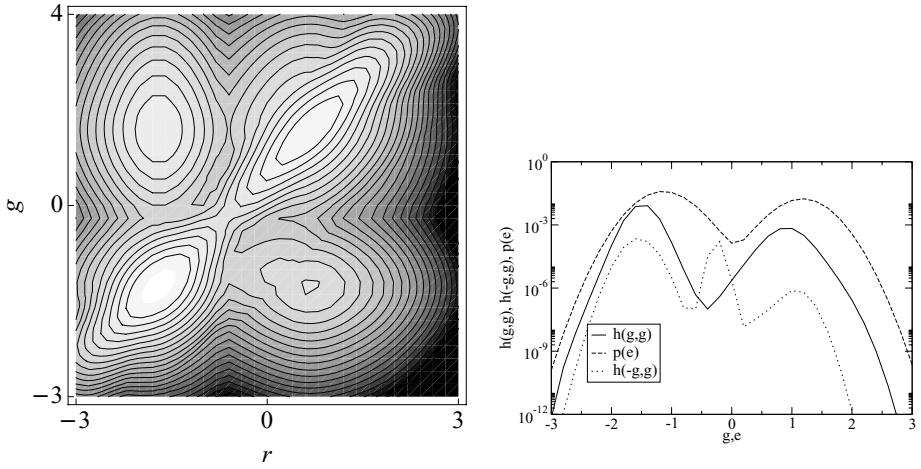

Figure 1. Left panel: Contour plot of the host population density on a logarithmic scale. Clearly visible along the $r = g$ diagonal are two peaks, centered around two coordinates (r_1, g_1) and (r_2, g_2), corresponding to two strains, driven into separation by pathogen–plant interactions. Two much weaker peaks with coordinates approximately (r_1, g_2) and (r_2, g_1), correspond to hybrid heterozygotes in the r and g genes. The subpopulation of necrotic hybrids lies below the $r = g$ diagonal, where $r > g$. The plot corresponds to the solution of equations (16) and (17) at a steady state ($t = 800$) for the following parameter values: $\beta = 1$, $\alpha = 1$, $\delta_I = 5$, $\delta_C = 1$, $\delta_P = 1$, $\sigma_I = 0.2$, $\sigma_{AI} = 0.2$, $\sigma_a = 0.8$, $\sigma_{M,r} = \sigma_{M,g} = \sigma_{M,e} = 0.1$, and $\sigma_r = \sigma_g = 1$. To avoid unrealistically high autoimmune and pathogen-induced host death rates, they were truncated by replacing $\exp \pm \left(\frac{r-g}{\sigma}\right)$ by $\min\left[\exp \pm \left(\frac{r-g}{\sigma}\right), 10^3\right]$. The steady state remains qualitatively unchanged for $0.6 \leq \sigma_a \leq 0.9$. Right panel: Plot of the host population $h(g, g)$ along the $r = g$ diagonal (solid line) and $h(-g, g)$ along $r = -g$ antidiagonal (dotted line) and of the pathogen population $p(e)$ (dashed line).

In Figure 1, each of the main maxima in the host plant distribution, located at (r_1, g_1) and (r_2, g_2), respectively, corresponds to an emerging host strain or subspecies with a distinct genetic makeup of guard and guardee proteins. Note that $r_1 \approx g_1$ and $r_2 \approx g_2$, which means that in each of the corresponding strains, the function of the guard and guardee proteins are geared toward both efficient immune response to the pathogen and low likelihood of autoimmune reactions. The equilibrium distribution in Figure 1 also shows two secondary peaks located approximately at (r_1, g_2) and (r_2, g_1). These peaks correspond to hybrids between the two emerging subspecies. These hybrids inherit their guard and guardee genes from parents of different subspecies and thus are either easy victims of pathogen attack (when $g > r$) or exhibit strong autoimmune necrosis (when $r > g$). Despite the equal per capita rate of birth of the homozygote and heterozygote offspring (see eq. 11), the peaks corresponding to the hybrids are much smaller due to their much higher death rates. This is illustrated in the right panel of Figure 1, which shows the bimodal equilibrium density distributions of the host (continuous line) and pathogen (dashed line) along the diagonal, as well as the trimodal density distribution of the host along the antidiagonal (stippled line). Here, the mode in the middle corresponds to the saddle between the two modes of the host distribution along the diagonal.

Obviously, the model can also produce other equilibrium distributions, depending on the values of model parameters, particularly the pathogen attack width. Examples are given in Figures 2 and 3. In Figure 2, the distributions converge to a unimodal equilibrium. In accordance with previous results (Doebeli and Dieckmann 2000; Dercole et al. 2003), this tends to happen for larger predator attack widths σ_a. In contrast, for small attack widths, the equilibrium distributions may have more than two modes along the diagonal, and hence more than two secondary hybrid peaks, as illustrated in Figure 3.

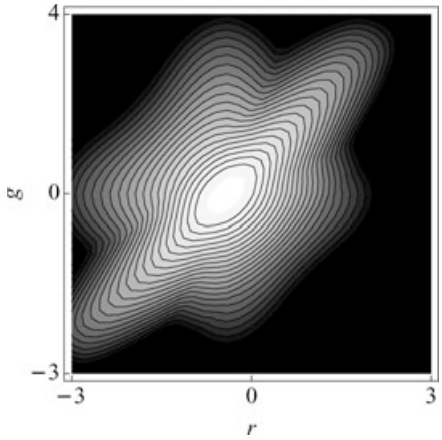

Figure 2. Same as Figure 1, but for a larger pathogen attack width, $\sigma_a = 1.2$. In this case, the host density does not evolve to a multimodal distribution, but forms a broad single peak, and the same is true for the pathogen density distribution (not shown).

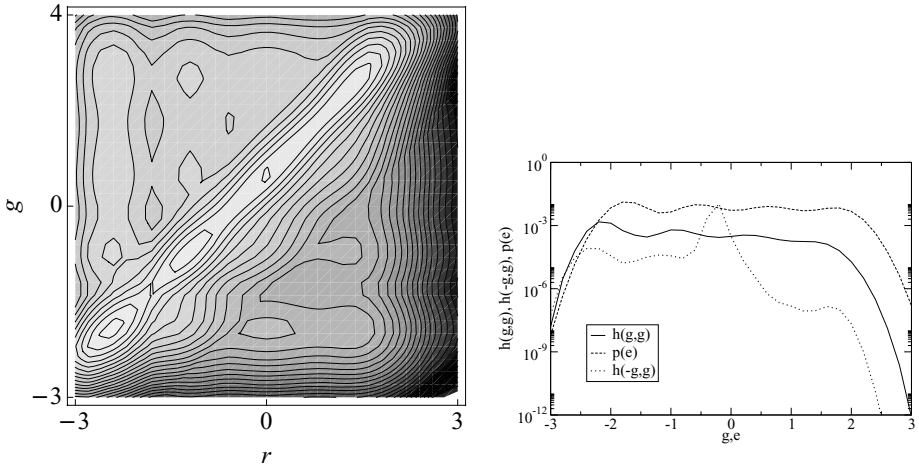

Figure 3. Same as Figure 1, but for a smaller pathogen attack width $\sigma_a = 0.4$. In this case, the host density evolves to a multimodal distribution, with multiple secondary peaks corresponding to hybrids (left panel). The multiple peaks are also evident if the host distribution is restricted to the diagonal, and the pathogen distribution also has multiple peaks (right panel). Along the antidiagonal, the host density distribution reflects the multiple secondary hybrid peaks (right panel).

Discussion

Both Bomblies and Weigel (2007) and Eizaguirre et al. (2009) recently argued that the immune system could play an important role in speciation processes. Although Eizaguirre et al. (2009) mainly considered the vertebrate immune system as a potential source of prezygotic reproductive isolation after divergent adaptation to pathogens, Bomblies and Weigel (2007) argued that defective autoimmune reactions in hybrids could be the source of postzygotic isolation. They were able to support this perspective by impressive experimental evidence in *A. thaliana* (Bomblies et al. 2007). Our aim here was to provide mathematical evidence for the feasibility of adaptive speciation due to host–pathogen interactions when isolation is caused by postzygotic autoimmune deficiencies.

Bomblies et al. (2007) have shown that hybrid necrosis, defined as a set of deleterious and often lethal phenotypic characteristics, can be caused by an epistatic interaction of loci controlling the immune response to attack by pathogens in *A. thaliana*. More precisely, hybrid necrosis is caused by detrimental activation of the plant immune system in the absence of pathogens, and Bomblies et al. (2007) showed that epistatic interactions between two loci are both necessary and sufficient for this form of hybrid necrosis. This corresponds to classic Dobzhansky–Muller incompatibilities (Gavrilets 2004), which we have modeled here by assuming that the optimal compromise between the ability to respond to a pathogen attack and the avoidance of undesired autoimmune reactions is attained by individuals with genotypes $(r, g \approx r)$, where r and g represent two loci controlling the immune response. Thus, two genotypes (r, r) and (R, R) with R very different from r can both be optimal, but their heterozygous hybrids (r, R) and (R, r) will suffer from a malfunctioning immune system. In the case of the plant immune system, the two genes r and g represent two different proteins (or groups of proteins)—"guard" and "guardee." Such proteins are thought to be central for the plant immune system (Jones and Dangl 2006), and their optimal functioning requires concerted evolution at both loci.

The model presented here combines a detailed, albeit schematic, description of the plant immune response based on the traits r and g, with the macroscopic, population-based representation of ecological interactions driving the evolution of these traits. The basic result is that for a range of intuitively appealing parameters, the model leads to adaptive speciation: as a result of frequency-dependent selection exerted by pathogen attack, a single ancestral host strain splits into two descending strains (r, r) and (R, R) that each have their distinct genetic makeup of the guard-guardee pathogen recognition system. Hybrids with guard and guardee genes coming from different parents either suffer necrosis due to autoimmune reaction, or they exhibit a weakened immune system due to a compromised ability to detect the pathogen attack. This latter form of hybrid disadvantage corresponds to the mechanism of postzygotic isolation due to MHC-based immune responses that was conjectured by Eizaguirre et al. (2009) to operate in vertebrates.

In contrast to most previous models of adaptive speciation, in which reproductive isolation is based on prezygotic mechanisms such as assortative mating, in our models reproductive isolation between diverging lineages emerges due to postzygotic hybrid inviability. Of course, a natural question would be whether such postzygotic isolation would select for prezygotic isolation in the form of assortative mating based on the immune system, as envisaged by Eizaguirre et al. (2009) (see also Gavrilets and Boake 1998; Gavrilets 2004). Thus, an interesting extension of our models would be to investigate the evolution of assortative mating

as a consequence of hybrid inviability due to autoimmune reactions. Our models could also be extended in a number of other ways. For example, it is known that many pathogens are capable of producing a number of different effector proteins that allow them to attack a host plant. Similarly, the immune system of the plant has a number of different ways of dealing with this effector variety. Accordingly, it would be interesting to see whether the type of diversification observed in our models would be easier or harder to obtain if the dimensionality of both the guard and guardee traits in the host and the effector trait in the pathogen is increased. For example, it is possible that hybrid necrosis, and hence postzygotic isolation, is increased if more than one guard-guardee pairs are involved in the immune response to a particular pathogen, because simultaneous incompatibility of various guards and guardees could lead to more severe autoimmune reactions.

Instead of considering larger numbers of guard-guardee pairs, another way to increase the complexity of the model would be to develop a more detailed, mechanistic description of the genetic network of activation and repression of the various pathways involved in the immune response based on a single guard–guardee pair. Clearly, it should be possible to come up with a number of alternative phenotype structures to model the mechanisms of the plant immune response, and it would be interesting to see whether such alternative models would give similar outcomes. In the present model, the genetic network regulating the immune response is assumed to be extremely simple in that it is assumed that the binding affinity of guard to guardee depends on the genetic distance ($r - g$). Ten Tusscher and Hogeweg (2009) have studied more traditional models for adaptive speciation based on resource competition under the assumption that the regulatory network determining the phenotypes important for competition are much more complicated, and realistic, than commonly assumed in such models. One of the main conclusion of these authors is that genetic complexity facilitates diversification and speciation, and it would be interesting to see whether similar conclusions would be reached if more genetic complexity would be incorporated into the models presented here. Another potentially interesting extension of the model would be to introduce diploid genetics, which would lead to a different type of mixing of phenotypes. In most models of adaptive speciation haploid and diploid models do not seem to generate substantially different outcomes, and it would be interesting to see whether this is also true for models presented here.

Despite being schematic and minimalistic, our model correctly reflects some of the main experimental observations of postzygotic isolation due to hybrid necrosis in *A. thaliana* (Bomblies et al. 2007, see also Appendix). Our model also predicts a class of hybrids with weakened immune response to pathogen attack, whose existence could be investigated in future experiments. Overall, we agree with Eizaguirre et al. (2009) that studying the role of the immune system for speciation processes is very promising. Adaptation in the immune system as a coevolutionary response to pathogens may be a potent mechanism for diversification based on both prezygotic and postzygotic reproductive isolation.

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Appendix

Here, we provide an argument for the fact that an increase in ambient temperature from 16°C to 23°C can indeed cause a biologically noticeable reduction in autoimmune reaction and hence rescue otherwise necrotic hybrids, as observed in the experiments of Bomblies et al. (2007). We make a simple estimate of how a change in temperature affects the Law of Mass Action governing the binding–unbinding equilibrium between the guard and guardee proteins. For simplicity, we assume that both proteins can exist only in two forms, free and bound to each other forming a dimer. We denote the free forms by R and G, and the dimer by RG. In the limit of weak binding (large dissociation constant k), i.e., when both proteins are mostly in free form, the concentration of the dimer is

$$[RG] = [R]_0[G]_0/k, \tag{A1}$$

where $[R]_0$ and $[G]_0$ are the total concentrations of proteins R and G. The temperature dependence of the dissociation constant is usually given by the Arrhenius form,

$$k = k_0 \exp\left(-\frac{E}{KT}\right). \tag{A2}$$

As a consequence, the relative decrease in the concentration of RG caused by the increase in temperature by ΔT is

$$\frac{[RG]_{T+\Delta T}}{[RG]_T} = \exp\left(-\frac{E\Delta T}{KT^2}\right). \tag{A3}$$

A cell can typically recognize a change in concentration larger than 20% [smaller shifts in concentrations are apparently perceived as "noise", Newman et al. (2006)]. Thus, for a very reasonable value of protein–protein dissociation energy $E \approx 8kT$, an increase in the temperature from 16°C to 23°C can produce a biologically meaningful decrease in concentration of the RG dimer. It is at least plausible that such a decrease can eliminate improper binding between R and G, and hence unwanted autoimmune reactions.