Plant immune system incompatibility and the distribution of enemies in natural hybrid zones
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Natural plant hybrid zones have been described as ‘sinks’ and ‘centers of biodiversity’ for herbivores and fungal pathogens. Jasmonic acid is known to be a critical signaling molecule for defense against these enemies. Does inhibition of jasmonic acid perhaps contribute to the susceptibility of hybrid plants to attack by herbivores? Here, we discuss recent evidence that plant immune system incompatibilities are likely to downregulate jasmonic acid (JA)-dependent responses through their effect on expression of the salicylic acid (SA)-dependent pathway. Because these hybrid immune incompatibilities are a function of environmental temperatures, they suggest a dependency between attack rates in hybrid zones and environmental conditions. Hybrid zones in colder environments, for example at higher elevations or latitudes, are more likely to exhibit elevated SA, suppressed jasmonic acid-dependent defenses, and lower resistance to herbivores and other necrotrophic pathogens.

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Introduction
As a consequence of their stationary existence, plants experience intense pathogen exposure and dramatic swings in temperature, water availability, and other abiotic factors. The ability of plants to recognize and selectively respond to this variation is essential for their survival in nature. Because defenses are costly [1], plants must be able to recognize benign microbes from serious pathogens, and trigger defenses only as appropriate to a particular situation [2]. This specificity of perception is accomplished in plants through the actions of nucleotide binding and leucine-rich repeat (NB-LRR) proteins which reside inside cells and perceive particular microbial effector molecules that advertise an active attack [3]. Following the perception of infection, these NB-LRR proteins trigger signaling cascades that result in the upregulation of defenses. One of the best studied of these is systemic acquired resistance (SAR), which depends on salicylic acid (SA) and the NPR1 gene, and is marked by upregulation of pathogen response genes, PR-1, PR-2, and PR-5 in Arabidopsis thaliana [4**]. The responses to infection can also trigger localized cell death, which prevents the spread of infection to adjacent plant tissues. Genotypes of Arabidopsis possess distinct suites of R genes, as well as different types of accessory proteins that assist the NB-LRR proteins at the top of the cascade [3–6]. What happens, then, when plants with different R genes or accessory proteins mate and produce offspring?

In exciting recent work, researchers have found that crosses can yield incompatible interactions among these R genes [7–11]. The importance of this finding as a mechanism for plant speciation has been recognized and reviewed elsewhere [12*,13*,14–16]. Our focus is quite different. Here, we assess how this finding may help explain a well-known pattern in the distribution of herbivores and other enemies of plants in nature. At the outset, it is important to note that herbivory or mechanical damage to plant tissues typically induces a separate defense pathway that is dependent on jasmonic acid (JA) and is suppressed by SA [4**]. Below, we first present the accumulated evidence that hybrid zones have greater abundance and diversity of herbivores. We then discuss the mechanistic basis of hybrid necrosis, showing that it, first, results from SA-dependent interactions at R gene loci and, second, is abolished with increasing temperatures, through the likely suppression of SA. Next, we discuss the evidence that immune incompatibility suppresses jasmonic acid-dependent responses [10**]. Finally, we discuss how suppression of jasmonic acid-dependent responses may explain the greater abundances of herbivores in plant hybrid zones.

Hybrid zones have greater abundance and diversity of herbivores
More than 100 studies have compared the herbivore community on plants in hybrid zones relative to the parental plants [17]. The patterns can be dramatic; in one particular hybrid zone for Populus fremontii × angustifolia, more than 85% of Pemphigus betae aphids were found on hybrids even though hybrids represented only 3% of available plants [18]. In a study of Eucalyptus, the average hybrid tree supported 53% more species of insect herbivores and fungi than did either parental
species [19]. It is not surprising, then, that Whitham and colleagues have described hybrid zones as ‘sinks for pests’ [18] and as ‘centers of biodiversity’ [19]. A meta-analysis of hybrid resistance to herbivores shows that of 9 studies testing hybrids under controlled common garden conditions, all found that hybrid offspring were more susceptible to herbivores than would be predicted by the susceptibilities of plants in the parental populations [17]. At least three hypotheses have been put forward to explain the greater abundance of herbivores on hybrid plants, including mixtures of defense compounds that are not as effective as those present in the parent plants [20], loss of vigor in hybrid plants [18], and increases in the seasonal duration of resource availability at hybrid zones [21]. In the following sections, we discuss recent evidence suggesting that downregulation of jasmonic acid should be considered as an alternative hypothesis. We further discuss how temperature influences this process and how it may influence hybrid zones in natural environments.

Hybrid necrosis as a function of SA and temperature

Hybrid necrosis results from SA-dependent epistatic interactions at R gene loci

Roughly 2% of intraspecific crosses between wild genotypes of Arabidopsis result in impaired progeny [8**]. Bomblies and colleagues determined that allelic variation at a single locus containing a tandem duplicate of two R genes (At5g41740 and At5g41750) was necessary and sufficient to cause hybrid necrosis in crosses between the Uk-1 and Uk-3 genotypes. To demonstrate the necessity of these genes for hybrid necrosis, they used artificial microRNA to silence At5g41740 and At5g41750, and found that crosses between the silenced lines then yielded normal offspring. They also transformed each allele into a common background (Col-0) and found that crosses between the silenced lines then yielded necrotic offspring, thus demonstrating that alleles at this locus were sufficient to generate the hybrid necrosis phenotype. Finally, they used microarray analysis to show that the effect of the R gene incompatibility was overexpression of defense-related pathway genes. In other words, offspring suffered autoimmune disorders, with the implication that overactivation of the SAR pathway was responsible.

Studies published this past year have consolidated and extended the importance of immune compatibility in intraspecific crosses in Arabidopsis [9**,10**,11]. It is now clear that at least some of these incompatible interactions involve modulation of SA [9**,10**]. Hybrid plants from incompatible crosses exhibit significantly elevated concentrations of SA [9**,10**]. Moreover, when parental lines are transformed to express a bacterial Salicylate Hydroxylase gene ( NahG) that converts SA to catechol, the offspring of crosses no longer exhibit necrosis. Likewise, the response is completely abolished in npr1 plants [9**]. In summary, the available data suggest that the hybrid necrosis phenotype is dependent on SA and the NPR1 gene.

Hybrid necrosis is suppressed at elevated temperatures

That elevated temperatures can abolish hybrid necrosis was discovered independently in interspecific crosses of wheat [22] and cotton [23] and has been described for interspecific crosses for tobacco [24–26], lettuce [27**], and pepper [28]. Elevated temperatures also abolish the hybrid necrosis of intraspecific crosses in Arabidopsis [8**,9**,10**,29**,30**]. In the cross between Uk-1 and Uk-3, hybrid necrosis and related stress signal transcription are completely absent at 23°C and above [8**]. For cotton hybrids, any temperature less than 35°C results in death by necrosis, whereas temperatures of 37–40°C abolish the necrotic phenotype [23]. Clearly, it is the relative, rather than absolute, growth temperature that is important (Figure 1). The mechanism by which high temperatures suppresses hybrid necrosis remains unknown. Below, we discuss the accumulating evidence that the necrotic phenotype is lost at elevated temperatures because of direct suppression of SA accumulation [29**].

Yang and Hua proposed their model to explain how interactions between plant R genes and modulators, such as BONZAI1 (BON1), can lead to suppression of SA production at elevated temperatures [29**]. At a growth temperature of 22°C, they observed constitutive expression of the Enhanced Disease Susceptibility_1 (EDS1) gene in wildtype plants. Loss of BON1 resulted in overexpression of defense and stunted plant growth at this temperature. BON1 was shown to negatively regulate an R gene, SNC1, which amplifies SA accumulation at 22°C. In contrast, at a growth temperature of 28°C, they found that EDS1 was completely suppressed in wildtype plants, even in the absence of functional BON1. This demonstrates that an SA feedback loop not only responds negatively to increasing temperature, but does so across temperatures that are well within those experienced by naturally occurring Arabidopsis [31**].

Figure 1

Temperatures yielding lethal necrosis (L) and normal growth (N) for available species and crosses. Data compiled from various sources [8**,10**,23,24,27].

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A number of additional studies have suggested that high temperatures are associated with low constitutive concentrations of SA and other phenolic compounds (Table 1). Again, the absolute temperature does not appear to be important, but rather the difference from the optimal growth temperatures. For tobacco, a temperature of \(24 \degree C\) or below is sufficient to stimulate production of SA [32]. Indeed, free and conjugated SA levels were barely detectable in tobacco grown at \(32 \degree C\) [33,34]. In contrast, *Arabidopsis*, which grows in cooler environments, mildly suppresses SA production at \(24 \degree C\) and stimulates SA production at temperatures of \(16 \degree C\) or below [10**].

Given that SA-dependent and JA-dependent responses are typically antagonistic [4**,35,36], what are the consequences of hybrid necrosis for expression of the JA-dependent pathway? In the following section, we address what is known about the JA-dependent responses in these hybrids.

**Jasmonic acid-dependent responses are suppressed in necrotic hybrids**

While upregulation of SA has been shown in numerous studies to repress jasmonic acid-dependent signaling [4**,35,36], Alcázar *et al.* recently provided the first documentation that such a response can occur because of immune incompatibility in hybrid plants [10**]. An important goal of their study was to understand whether epistatic interactions among genes were differentially influenced within *Arabidopsis* growing at \(14 \degree C\) or \(20 \degree C\). They tested recombinant inbred lines (RILs) from

![Figure 2](image_url)
crosses between Ler and each of six accessions, and found that some RILs from the Ler × Kas-2 cross yielded a particularly strong dwarfing phenotype at the low temperature, which was absent at the high temperature. These dwarfed RILs had strong constitutive expression of SA-dependent response, including high transcript levels of PR-1. Furthermore, the dwarfed RILs exhibited significantly reduced transcript levels of PDF1.2 relative to the parent lines, especially at low temperatures. To demonstrate a clear role of R gene incompatibility, they expressed a group of TIR-NB-LRR genes from one locus of one parent (Ler) in the other parent background (Kas-2), and found that this was sufficient to induce strong upregulation of SA and suppression of JA-dependent responses. Thus, this study was able to draw an important connection between SA and JA levels in the context of epistatic interactions likely to involve R genes.

**Implications of immune incompatibility for natural hybrid zones**

The importance of jasmonic acid-dependent responses in the defense of plants against herbivores is well-established [37,38]. Nevertheless, the fact that hybrid plants in nature suffer disproportionately large herbivore loads has not been previously linked to possible immune incompatibility-based upregulation of SA, and its repercussions for jasmonic acid-dependent defense responses. Indeed, the absence of a comparative assessment of salicylic acid and jasmonic acid concentrations across natural hybrid zones represents a significant gap in our knowledge. In the simplest model, constitutive SA concentrations and herbivore densities would be predicted to be highest for hybrid plants, whereas jasmonic acid concentrations would be highest in the parental lines (Figure 2a). For hybrid zones that are parallel to a temperature gradient, the expectation would be stronger upregulation of constitutive SA and higher herbivore densities at the cold end of the temperature gradient, unless the lower temperatures directly suppress herbivore densities (Figure 2b). Last, if high temperatures suppress the immune incompatibility phenotype, then hybrid zones at lower latitudes and lower elevations would be expected to have lower constitutive levels of SA and higher constitutive concentrations of jasmonic acid (Figure 2c). While there is support for an increase in plant concentrations of SA and other phenolic compounds with increasing elevation (Table 2), the distribution of jasmonic acid or other fatty acids across any natural gradient remains unknown.

**Conclusions**

Natural plant hybrid zones have been described as ‘sinks’ and ‘centers of biodiversity’ for herbivores and fungal pathogens. Jasmonic acid is known to be a critical signaling molecule for defense against these enemies, but this hormone has only recently been linked to hybrid zones. New evidence shows that in some cases the hybrid necrosis is because of R-gene incompatibilities that are dependent on SA and lead to suppression of jasmonic acid-dependent responses. The suppression of jasmonic acid-dependent responses provides a new mechanism to explain the greater abundance and diversity of herbivores on hybrid plants. This hypothesis remains to be tested. The paucity of data regarding SA and jasmonic acid concentrations across natural hybrid zones represents a significant gap in our knowledge. Likewise, the frequency of immune incompatibilities relative to other incompatible epistatic interactions [53] in natural plant hybrid zones remains unknown. Suppression of the hybrid necrosis phenotype at moderate ambient temperatures suggests that immune incompatible hybrids will survive and reproduce in nature under realistic environmental conditions. Furthermore, they are likely to survive differentially across the landscape with greater prevalence in warmer climates.

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**References and recommended reading**

Papers of particular interest published within the period of review have been highlighted as:

- of special interest
- **of outstanding interest**


This paper is the first to report that hybrid necrosis can occur because of R gene-based incompatibilities, involves upregulation of the SAR pathway, and is suppressed at elevated temperatures.


This paper is one of only two studies that have measured SA concentrations in the tissues of hybrid plants to show that immune incompatibility is involved in the hybrid necrosis response.


This paper is the first to report that jasmonate-dependent responses are suppressed in a hybrid plant. They used recombinant introgressed lines for a cross between the Ler and Kas-2 accessions of Arabidopsis and found that some of these RILs exhibited defective immune responses.


An elegant and thorough study of natural selection favoring different phenotypes in Mimulus guttatus in coastal and inland habitats. This study is notable for the extensive sampling of genotypes across the geographic range and for its excellent statistical analysis.


This paper provides new insight into the protein-protein interactions at the top of the R gene surveillance system.


This paper provides one of the first models to explain how high temperatures may control an SA accumulation loop in Arabidopsis.


This paper clarifies the interactions between important R genes and associated proteins at the top of the SA-dependent signaling cascade.


One of the first assessments of seed mortality and other demographic factors for Arabidopsis thaliana natural populations along an elevational gradient.


This paper documents a strong and novel hybrid necrosis response that results from incompatible alleles of genes regulating the biosynthesis of histidine.