Pollen-Pistil Interactions and Their Role in Mate Selection[1][OPEN]

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A PLACE FOR BOYS AND GIRLS TO MEET IN THE PLANT WORLD: THE PISTIL IS THE VENUE FOR MATE SELECTION

As a group, the flowering plants have remarkably diverse modes of reproduction that run the gamut from clonal propagation to obligate outcrossing. Articles in this volume focus on sexual reproduction, and there is great diversity even within this slice of the reproduction spectrum. Like all diverse systems, reproductive characters are the end results of molecular and genetic mechanisms that generate diversity. Selective pressures that act upon this diversity lead to the differentiation of lineages and the maintenance of their identity. The seemingly minuscule slice of the angiosperm life cycle represented in the postpollination to prezygotic phase, which is dominated by pollen-pistil interactions, plays a large role in these evolutionary processes.

The pistil is unique to angiosperms. It serves a protective role and functions as a conduit for pollen tubes to grow to the ovary, but it also provides a venue for pollen-pistil interactions that regulate pollen tube growth and, hence, fertilization. Although the earliest diverging angiosperm lineages do not possess the full set of canonical angiosperm pistil traits (Endress and Doyle, 2009; Endress, 2011, 2015; Lora et al., 2016), the core lineages (which display the most diversity) have a stigma specialized for the receipt of pollen and a style that pollen tubes must traverse before fertilization can occur. Some authors attribute angiosperm diversity to phenomena that depend on pollen-pistil interactions taking place in stigmas and styles, such as pollen competition and self-incompatibility (SI; Mulcahy and Mulcahy, 1988; Williams, 2012). Therefore, the evolved state of the pistil, as the organ that stands between pollen receipt and the ovule, allows it to assume a gatekeeper function, facilitating the growth of desirable pollen tubes and discouraging invaders and less desirable pollen (Heslop-Harrison, 2000).

Our focus is on pollen-pistil interactions that contribute to mate selection in the domains of SI and interspecific pollen rejection. Together, these processes define a window of genetic relationships for successful mating: SI prevents mating between very closely related individuals of the same species, and interspecific pollen rejection prevents crosses that are too distant. SI clearly contributes to a species' long-term success, as SI lineages are genetically diverse and very persistent (Goldberg et al., 2010; Goldberg and Igić, 2012). However, transitions from SI to self-compatibility (SC) also are important. SC lineages emerge frequently and may

ADVANCES

- Nonself recognition: Collaborative nonself recognition between S-RNase and SLF provides a new way of thinking about S-RNase-based SI.
- *Papaver* SI is transferrable. Expressing Prp-S and Prs-S in *Arabidopsis thaliana* introduces SI into this otherwise SC species. Though accomplished earlier using Brassicaceae SI genes, the *Papaver* results raise the possibility of manipulating compatibility in a broad range of species through genetic engineering.
- SI and UI share factors. In Solanaceae, both pistil and pollen side SI genes are shared between interspecific and intraspecific pollination mechanisms. It has long been known that transitions from SI to SC are frequent during plant evolution, but the potential for broader changes in compatibility that arise from coupling these systems has not been widely appreciated.

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make range expansion possible under conditions of mate limitation (Baker, 1955, 1967; Pannell et al., 2015) but SC lineages also may be vulnerable to extinction due to inbreeding depression. A species with any substantial level of outcrossing entertains the risk that the stigma will be challenged with pollen from other species. The ability to recognize and reject heterospecific pollen, therefore, is important to prevent wasted ovules as well as to maintain species integrity. Outcrossing species in complex plant communities often are challenged by heterospecific pollen (Arceo-Gómez and Ashman, 2014) and, thus, would be expected to maintain robust interspecific pollen recognition and rejection mechanisms.

A limited number of SI and interspecific pollen rejection mechanisms have been elucidated by genetic and biochemical analyses. We favor a synthetic view emphasizing the pistil's overall mate selection function. This perspective provides a framework in which to view a range of pollen-pistil interactions that can function in both the generation and maintenance of species.

INTRASPECIFIC REPRODUCTIVE BARRIERS: SI

SI is a genetically controlled system for recognizing and rejecting self-pollen in plants with hermaphroditic flowers (de Nettancourt, 1997, 2001; Franklin-Tong, 2008). SI enforces outcrossing, but it is distinct from dioecy or phenological barriers to self-fertilization. The term is applied generally to genetically controlled systems that prevent selfing, and a variety of SI systems have evolved across the angiosperms. Because the simple genetics of SI provided a starting point for analyses, SI systems are the best understood pollen-pistil interactions at the molecular and genetic levels and, thus, provide paradigms for understanding interspecific pollen rejection.

Genetic control of SI usually derives from a single polymorphic locus called the *S*-locus. Although the *S*-locus has the same name in different families, the locus structure, the nature of the encoded genes, and the mechanisms of SI may be completely different (de Nettancourt, 2001). Heteromorphic SI, where alternate floral morphs are produced (e.g. long- and short-style *Primula vulgaris*; Darwin, 1877; Gilmartin and Li, 2010), is common, but outcrossing is achieved by structural barriers and not pollen-pistil interactions. In SI species with homomorphic flowers, the *S*-locus encodes pistil-expressed genes that provide for the recognition and rejection of self-pollen or the favoring of nonself pollen. Homomorphic SI is described as gametophytic if pollen compatibility is determined by its own *S*-haplotype and sporophytic if the compatibility of the haploid pollen is determined by the diploid sporophyte that produced it. In either case, the *S*-locus encodes genes controlling specificity on both the pollen and pistil sides. The genes and their functions have been studied extensively in three distinct SI systems (de Nettancourt, 1997, 2001; Franklin-Tong, 2008).

SI MECHANISMS: BRASSICACEAE

Sporophytic SI has been studied intensively in Brassicaceae. In simple cases where dominance does not play a role, sporophytic SI is quite selective, since a compatible pollination requires that there are no *S*-haplotypes in common between male and female partners (de Nettancourt, 1997, 2001; Franklin-Tong, 2008). The Brassicaceae pistil consists of a dry stigma with numerous papillar cells for pollen reception. Compatible pollen germinates on the papillar cell surface, and pollen tubes traverse a short transmitting tract before entering the ovary. Therefore, it is not surprising that SI pollen rejection is a rapid response occurring on the stigmatic papillar cells. The Brassicaceae SI system was one of the first to be successfully studied at the molecular level (Nasrallah and Wallace, 1967; Nasrallah et al., 1985). It is now established that each *S*-haplotype includes an allele of the *S*-locus receptor kinase (*SRK*) gene expressed in the stigma and a matching allele of an *S*-locus Cys-rich (*SCR*) gene expressed in the diploid tapetum of the anther, such that the encoded SCR protein is deposited in the exine of haploid pollen grains (Stein and Nasrallah, 1993; Schopfer et al., 1999; Takayama et al., 2000). SRK and SCR form a receptor-ligand pair that initiates self-pollen rejection. In an incompatible SI interaction in *Brassica* spp. and *Arabidopsis*, the SCR protein diffuses from the pollen exine and interacts with the SRK extracellular domain, which is located in the plasma membrane of the stigmatic papillar cells. SRK signals to intracellular proteins in the papillar cell, and downstream events, including protein phosphorylation and changes in intracellular calcium, lead to the rejection response (Takayama and Isogai, 2005; Nasrallah and Nasrallah, 2014; Iwano et al., 2015). In compatible pollinations, independent of SRK signaling, the papillar cells are stimulated to secrete material that presumably supports pollen tube growth (Safavian and Goring, 2013). In the incompatible response, a key secretory protein, exo70A1 (exocyst complex component 70A1), is destabilized, and secretory vesicles are targeted to a vacuole rather than secreted (Indriolo and Goring, 2014). Since diffusion of the pollen SCR polypeptide ligand to the SRK receptor induces the rejection response in the papillar cell, SC can be caused by a loss-of-function mutations in either pollen-expressed *SCR* or pistil-expressed *SRK* (Dwyer et al., 2013).

SI MECHANISMS: *PAPAVER* SPP.

Papaver rhoeas has a gametophytic SI system that also relies on receptor-ligand signaling and an induced rejection response. However, the *S*-specific polypeptide ligand is produced in the stigma and induces a rejection response in incompatible pollen tubes. Gametophytic SI is inherently less selective than sporophytic SI because it is the *S*-haplotype of the haploid male gametophyte that determines compatibility (de Nettancourt, 1997, 2001; Franklin-Tong, 2008). The pistil-side *S*-genes

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encode secreted Prs-S proteins, and the corresponding pollen-side genes encode Prp-S proteins that are located at the plasma membrane (Foote et al., 1994; Wheeler et al., 2010). Poppies have an unusual stigma with specialized spoke-like rays that receive pollen; although the anatomy differs from Brassicaceae in almost every detail, a common element is that pollen tubes only grow a short distance before entering the ovary. The SI response in *Papaver* spp. is likewise very rapid, and arrest of incompatible pollen tube growth occurs on the stigma.

Papaver SI is better understood at the physiological level than any other system, in part, because the response can be replicated faithfully in vitro (Franklin-Tong et al., 1988). In vitro systems have been used successfully to elucidate pollen tube cell biology and pollen tube attraction (Cheung et al., 1995; Palanivelu and Preuss, 2006; Okuda et al., 2009; Kanaoka and Higashiyama, 2015), but *P. rhoeas* is unique among pollen rejection systems because the SI response also can be reproduced faithfully. The addition of incompatible Prs-S protein to pollen tube growth medium rapidly initiates a sequence of events, including cessation of growth, changes in calcium currents and pH, and fragmentation of the actin cytoskeleton in pollen tubes (Franklin-Tong, 2008; Wilkins et al., 2014, 2015). Over longer time periods, a programmed cell death response is initiated, and pollen tube growth inhibition is irreversible (Bosch and Franklin-Tong, 2007, 2008). Strikingly, incompatible Prs-S protein alone is sufficient to induce this series of responses, and the Prp-S protein is sufficient to transduce the signal (Foote et al., 1994; de Graaf et al., 2012). While other SI systems require modifier genes (i.e. non-*S*-locus genes that are required for full function) on either the pollen or the pistil side, or both, *Papaver* SI appears to be simpler. The initial signaling to Prp-S in incompatible pollen tubes may directly affect intracellular calcium, leading to a complex but conserved series of events with cell death as the end point (Wilkins et al., 2014, 2015).

The proposition that Prs-S and Prp-S function together as a ligand-receptor signaling module that signals to a conserved cell death response has great potential significance. This hypothesis was tested recently by transferring the *P. rhoeas* SI system to Arabidopsis. Lin et al. (2015) showed that expressing *Prs-S_1* in Arabidopsis papillar cells and *Prp-S_1* in pollen (i.e. an incompatible *Prs-S/Prp-S* pair) results in SI (Lin et al., 2015). Given the phylogenetic distance between *P. rhoeas* and Arabidopsis, this exciting result suggests that the *Papaver* SI system may be used to manipulate compatibility in virtually any species, which has long been a goal of plant biotechnologists (see Advances).

S-RNASE-BASED SI

Species that display S-RNase-based SI have wet stigmas and long styles, and incompatible pollen tube rejection generally occurs in the style well after pollen tube germination (Fig. 1). S-RNases function as both pistil-side recognition proteins and as cytotoxins in SI, and their recognition function determines *S*-specificity on the pistil side. Allelic S-RNase sequences often show striking levels of divergence; for example, S_{A2}- and S_{C10}-RNase from *Nicotiana alata* show only 43% identity (McClure et al., 2000). *S*-specificity has been investigated by in vitro mutagenesis and domain-swap experiments (Kao and McCubbin, 1996; Matton et al., 1997; Zurek et al., 1997) with divergent results. Matton et al. (1997) converted S_{11}-RNase into a chimeric sequence recognized as S_{13}-RNase in *Solanum chacoense* by exchanging four residues between this pair of highly similar proteins. Intriguingly, exchanging just three residues results in a dual-specificity protein that caused the rejection of both S_{11} and S_{13} pollen (Matton et al., 1999). More recently, Soulard et al. (2013) generated another dual S_{11}-/S_{13}-RNase by incorporating an *N*-glycosylation site into a variable region. In contrast, 11 domain-swap experiments in *Nicotiana* and *Petunia* spp., where sequence segments ranging from 15 to 189 residues were exchanged, always resulted in chimeric S-RNases that failed to be recognized at all (Kao and McCubbin, 1996; Zurek et al., 1997). Thus, while S-RNases encode *S*-specificity information, it is not always localized to a specific region of the protein. As we discuss later, it is not completely clear how S-RNase specificity is perceived on the pollen side.

S-RNase conserved regions are crucial for their cytotoxic activity. Early studies identified five conserved regions, C1 to C5, that together account for about 30 of the approximately 200 residues in a typical S-RNase (Anderson et al., 1989; Ioerger et al., 1991). Conserved regions C2 and C3 each contain a His residue important for RNase activity (Kawata et al., 1988; McClure et al., 1989). The observation that pollen RNA is specifically degraded after self-pollination led to the suggestion that S-RNases function as *S*-specific cytotoxins in SI (McClure et al., 1989, 1990; Gray et al., 1991; Huang et al., 1994; Kowyama et al., 1994). Although there is considerable evidence that SI in Solanaceae is based on S-RNase cytotoxicity, recent evidence suggests that other effects also may be important. For example, Roldán et al. (2012) reported the disorganization of F-actin in incompatible *N. alata* pollen tubes, raising interesting parallels to *Papaver* SI. Moreover, there have been suggestions that S-RNase-based SI in *Pyrus* spp. (Rosaceae) involves a complex set of physiological responses, including a cell death response, reactive oxygen species, and the actin cytoskeleton (Liu et al., 2007; Wang et al., 2009, 2010). Although these *Pyrus* studies often rely on in vitro experiments that have not been thoroughly validated in vivo, there is good evidence that S-RNase-based SI in *Prunus* spp. differs fundamentally from that in Solanaceae (Tao and Iezzoni, 2010; Sassa, 2016). Thus, it is best to remain open to the possibility that S-RNase may have a series of effects on incompatible pollen tubes. At present, it is impossible to distinguish between direct effects of SI and indirect effects of reduced pollen tube growth or physiological

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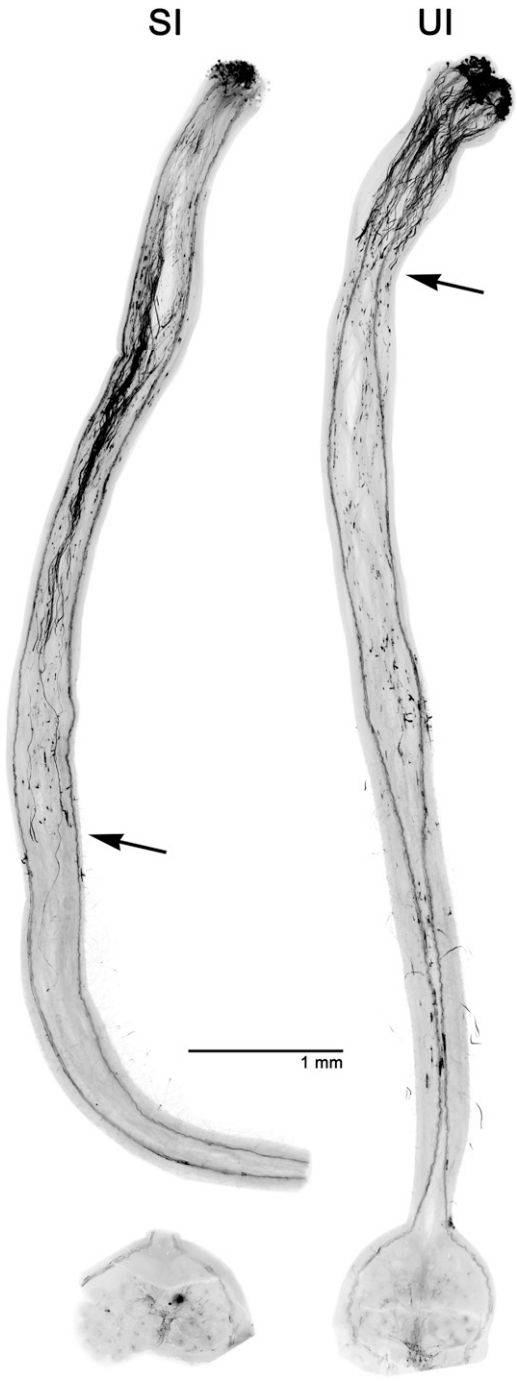

Figure 1. Rapid interspecific unilateral incompatibility (UI) pollen rejection compared with SI. Pistils stained with Aniline Blue fluorochrome are shown. Left, Self-pollinated SI *Solanum chilense* LA2884. Right, SI *S. chilense* LA2884 pollinated by *S. lycopersicum* 'VF36'. The UI rejection response in this example occurs closer to the stigma and is typical of crosses between SI green-fruited species and SC red-fruited species (Baek et al., 2015). Arrows indicate the position in the style where fewer than three pollen tubes pass at 48 h after pollination (compatible pollen tubes reach the ovary after 24 h). Images are by You Soon Baek and Suzanne Royer.

decay in a dying pollen tube. Robust in vivo experiments are needed to determine the temporal series of events and distinguish between potential mechanisms in incompatible pollen tubes.

Although S-RNases are the crucial pistil-side determinants of *S*-specificity and also are involved directly in self-pollen rejection, they are not sufficient for pistil-side SI function. Three modifier genes have been identified that contribute to SI but that do not determine *S*-specificity (Fig. 2). Loss-of-function studies show that the Asn-rich HT proteins are required for SI in *Nicotiana*, *Petunia*, and *Solanum* spp. (McClure et al., 1999; O'Brien et al., 2002; Puerta et al., 2009). Most SI *Solanum* spp. express two *HT* genes designated *HT-A* and *HT-B* (Kondo et al., 2002; O'Brien et al., 2002). For example, with the exception of *Solanum habrochaites*, which expresses only an *HT-A* gene, the green-fruited species in the tomato clade express both *HT-A* and *HT-B* (Covey et al., 2010). These green-fruited species are predominantly SI or have recently undergone a shift in their mating system to SC. In contrast, cultivated tomato (*Solanum lycopersicum*) and its SC red- or orange-fruited relatives (*Solanum galapagense*, *Solanum cheesmaniae*, and *Solanum pimpinellifolium*; here referred to as red-fruited species) do not express HT proteins (Kondo et al., 2002). Interestingly, HT proteins are preferentially degraded after compatible pollination in *Nicotiana* spp. (Goldraij et al., 2006). The 120-kD glycoprotein (120K), another SI modifier, is an abundant S-RNase-binding protein located in the *N. alata* transmitting tract (Lind et al., 1994, 1996; Cruz-Garcia et al., 2005) that also is required for *S*-specific pollen rejection (Hancock et al., 2005). More recently, NaStEP (*N. alta* Stigma Expressed Protein) was identified as a proteinase inhibitor that is required for SI and also affects HT protein stability (Busot et al., 2008; Jiménez-Durán et al., 2013). Further insights into the functions of HT, 120K, and NaStEP are needed.

S-locus F-box (*SLF*) genes were first identified as pollen-expressed F-box protein-encoding genes linked to S-RNase in *Antirrhinum* spp. Because they showed low sequence polymorphism, *SLF* genes were not at first thought to function as pollen *S*-specificity determinants (Lai et al., 2002). However, Sijacic et al. (2004) confirmed an association with SI by a gain-of-function experiment in *Petunia inflata*. Recent transcriptome studies suggest that an array of 16 to 20 *SLF* genes function in SI in *Petunia* spp. (Box 2; Williams et al., 2014a; Kubo et al., 2015). Consistent with this finding, the genome sequence of SC *Solanum pennellii* LA0716 (a recently derived SC accession of an otherwise SI species) contains an array of 23 *SLF* genes, while the SC tomato genome shows, at most, four intact genes (Bolger et al., 2014; Li and Chetelat, 2015).

The SLF proteins are thought to function in SI as components of SCF complexes (SCF^{SLF}), and the *Petunia* complexes have been the most thoroughly characterized. Li et al. (2014) recently expressed tagged SLF and recovered associated Rbx1-like, Skp1-like, and Cullin1-P (CUL1-P) proteins by coimmunoprecipitation. A reciprocal pull-down assay where the Skp1-like

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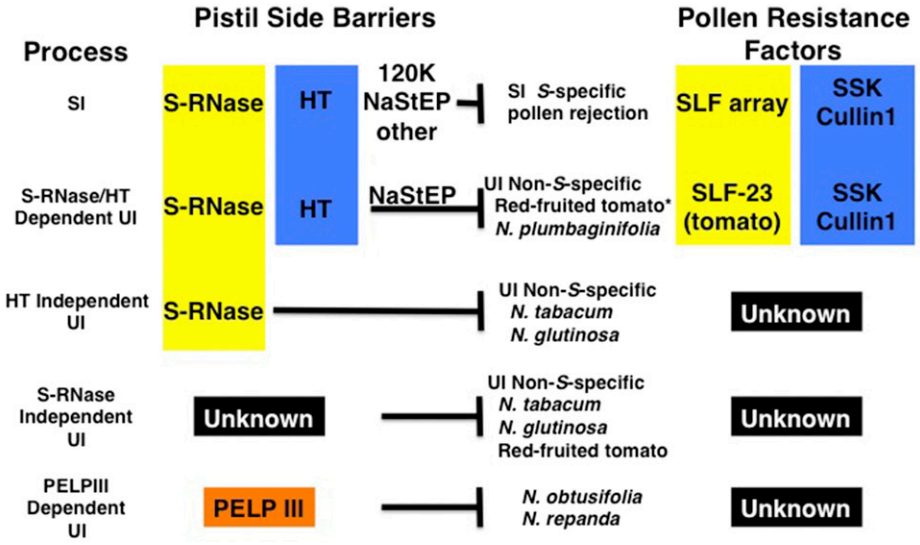

Figure 2. Pollen rejection mechanisms in Solanaceae. At least five distinct processes can be recognized. The intraspecific S-RNase-dependent SI mechanism is the best characterized. SI genes are implicated in some interspecific UI mechanisms (yellow, SI genes that determine specificity; blue, SI modifier genes). It is especially noteworthy that three distinct processes are S-RNase dependent but use different modifier genes. Not all pollen and pistil factors have been identified in all species studied. Pollen-side UI factors have only been identified in tomato relatives. Only a single *SLF* gene, *SLF-23*, is known to function in UI. *, The requirement for a tomato equivalent of NaStEP is inferred from the similarity to *N. plumbaginifolia* pollen rejection but has not been confirmed experimentally. S-RNase-independent mechanisms function in several examples of interspecific UI pollen rejection. Several such mechanisms probably exist but are lumped together because they are undefined at present. One S-RNase-independent mechanism that has been characterized relies on PELPIII (Pistil Extensin-Like Protein III, orange).

protein, PiSSK1 was tagged provided evidence for multiple SCF^{SLF} complexes (Li et al., 2014). This is consistent with the suggestion that each member of the SLF protein array binds individually to SSK and CUL1-P to form a series of SCF^{SLF} complexes that function together in SI (Kubo et al., 2010, 2015; Entani et al., 2014; Williams et al., 2014b). Several gain-of-function studies support the role of individual *SLF* genes in determining pollen-side *S*-specificity (Sijacic et al., 2004; Kubo et al., 2010, 2015). Therefore, the genes encoding the common SCF complex components that do not contribute to *S*-specificity (e.g. *PiSSK* and *PiCUL1-P*) are pollen-side modifier genes (Fig. 2). In the collaborative nonself recognition model (see Advances), a collection of SLF proteins brings all nonself S-RNases into the array of SCF^{SLF} complexes for ubiquitylation and subsequent degradation (Qiao et al., 2004; Zhao et al., 2010; Entani et al., 2014; Williams et al., 2015).

The collaborative nonself recognition model is attractive, but other processes also may contribute to SI. Goldraij et al. (2006) showed that S-RNases are taken up by both compatible and incompatible pollen tubes and targeted to vacuoles. In incompatible pollen tubes, the endomembrane system eventually breaks down, and S-RNases are released into the pollen tube cytoplasm, while in compatible pollen tubes, S-RNases remain compartmentalized. Interestingly, S-RNase uptake and compartmentalization are not affected in *HT*-suppressed plants (Goldraij et al., 2006), so HT protein likely functions downstream of these processes. Either compartmentalization or degradation could be equally effective in preventing S-RNase cytotoxicity, but the relative contributions from these processes to SI are not clear. One suggestion is that the processes operate in parallel (McClure, 2009; Williams et al., 2015).

The hypothesis that pollen-side *S*-specificity is determined by nonself recognition through a collection of SLF proteins (rather than self-recognition by a single protein) requires a shift in thinking about specificity itself (Box 1). Under this model, each SLF protein is thought to recognize one to four nonself S-RNases, such that the collection of SLF proteins encoded by a given *S*-haplotype recognizes all S-RNases except the self protein (Kubo et al., 2010, 2015; Iwano and Takayama, 2012; Williams et al., 2015). The extensive transcriptome analysis and functional testing presented by Kubo et al. (2015) provide strong support for this model. However, it is worth revisiting results from S-RNase mutagenesis studies investigating pistil-side specificity. Under the nonself recognition model, a novel S-RNase that is not recognized by the SLF array in a given *S*-haplotype should cause pollen rejection (because no SLF protein is available to provide resistance). This is concordant with results from in vitro mutagenesis experiments in *S. chacoense*, where exchanging four residues between S_{11}- and S_{13}-RNase switched *S*-specificity (i.e. the S_{11}/S_{13} chimera caused rejection of S_{13} pollen but not S_{11} pollen; Matton et al., 1999) but exchanging just three residues resulted in an S-RNase recognized in pollen as both S_{11}

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BOX I: NONSELF RECOGNITION IN SOLANACEAE

SI in Solanaceae is thought to be unique because it depends on nonself recognition. In effect, SI subdivides a population into a series of mating types whose cross compatibility or incompatibility is determined by the *S*-genotype. *Papaver* and Brassicaceae SI is characterized by self-recognition mediated by receptor-ligand interactions; while the details are unique in each system, at the most basic level, the ligand-receptor interaction induces incompatibility. Kubo et al. (2010, 2015) suggest that Solanaceae SI, in contrast, should be understood in terms of the nonself recognition of S-RNases by SLF proteins. This is a different way of understanding *S*-specificity. Under nonself recognition, rather than recognize a single (i.e. self) haplotype and target it for rejection, a given *S*-haplotype must recognize *S-RNase* alleles encoded by all other *S*-haplotypes. Thus, in this model, it is the failure to recognize self that causes incompatibility. Since an *S*-haplotype potentially must recognize a large number of nonself S-RNases, a further implication (and challenge) is that *S*-specificity cannot be understood without reference to a large number of *S*-haplotypes.

Kubo et al. (2015) performed an elegant analysis of *SLF* genes from eight *Petunia S*-haplotypes and provide insight into how nonself recognition could work. The 168 sequenced genes are classified into 18 types by sequence similarity (i.e. *SLF1*, *SLF2*, *SLF3*, etc.). Each *S*-haplotype contains 16 to 20 *SLF* genes, each belonging to one of the 18 types. For example, the S_x-haplotype encodes an array of *SLF* genes designated S_x-*SLF1*, S_x-*SLF2*, etc., and the S_y-haplotype encodes alleles S_y-*SLF1*, S_y-*SLF2*, etc. Alleles such as S_x-*SLF1* and S_y-*SLF1* show varying degrees of sequence similarity. The variability in gene number (i.e. 16–20) arises because a given *S*-haplotype may have zero to two copies of each *SLF* type.

Kubo et al. (2010, 2015) propose that, together, the array of *SLF* genes encoded by a particular *S*-haplotype recognize all S-RNases except self S-RNase. This possibility could be explained if each *S*-haplotype is missing the *SLF* type that recognizes self S-RNase or if the *SLF* type is so divergent that it cannot function. This logic leads to specific predictions about which *SLF* types recognize specific S-RNases. Kubo et al. (2015) tested many of these predictions in gain-of-function experiments by transforming plants with functional *SLF* alleles and found that they gain compatibility as predicted. The results provide support for nonself recognition. Apparently, each *S*-haplotype encodes sufficient *SLF* alleles to recognize all S-RNases in a population.

and S_{13} (i.e. caused the rejection of both S_{13} pollen and S_{11} pollen; Matton et al., 1999). This result can be explained if one or more SLF proteins in the S_{11} array recognize the former chimera but the latter is not recognized by any SLF in either the S_{11} or the S_{13} array. In contrast, the *Petunia* and *Nicotiana* S-RNase domain-swap experiments are not as easily accommodated (Kao and McCubbin, 1996; Zurek et al., 1997). It seems reasonable that exchanging 15 to 189 residues between allelic proteins should result, at least sometimes, in novel S-RNases that would not be recognized by the SLF array and, therefore, cause pollen rejection. However, none of the 11 chimeric S-RNases tested caused pollen rejection. Trivial explanations are unlikely because each of the nine *Nicotiana* spp. chimeras retain RNase activity (Zurek et al., 1997) and at least one functions in interspecific pollen rejection (Beecher et al., 2001). Under the collaborative nonself recognition model, the S-RNase domain-swap results could be explained if one or more SLF proteins recognized each of these 11 novel chimeric S-RNases. However, the functional testing reported so far suggests that most SLF proteins recognize a fixed set of S-RNases (Kubo et al., 2010, 2015). More information about how S-RNases are recognized by SLF proteins is needed to understand how the SLF array can recognize novel proteins.

INDUCED POLLEN REJECTION VERSUS CONSTITUTIVE BARRIER/RESISTANCE

Papaver and Brassicaceae SI are both systems characterized by a rapidly induced rejection response mediated by a ligand-receptor interaction. In Brassicaceae, signaling initiates the rejection response in the stigma papillar cell, while in *Papaver*, it is the pollen tube that responds to signaling. As the rejection response is induced in these systems, loss-of-function mutations in either the pollen- or pistil-side genes can cause SC, and, with some notable exceptions (Takada et al., 2013), the default for intraspecific pollination is compatibility. Iwano and Takayama (2012) refer to *Papaver* and Brassicaceae SI as self-recognition systems in the sense that the pollen- and pistil-expressed protein pair, encoded in a common (i.e. self) *S*-haplotype such as *S*-haplotype pairs of Prs-S/Prp-S and SCR/SRK proteins (i.e. self-recognition), interact to induce rejection.

SI in Solanaceae is fundamentally different from SI in either Brassicaceae or *Papaver*. In Solanaceae, pollen-side SI genes from a given *S*-haplotype recognize all pistil-side genes except the self gene (i.e. nonself recognition). SI in Solanaceae is not based on ligand-receptor signaling in the traditional sense; instead, pistil-side functions amount to a barrier to pollination, and pollen-side functions selectively provide resistance to nonself barriers. The key barrier proteins in SI Solanaceae spp. are pistil-expressed S-RNases (McClure et al., 1989), and the pollen-resistance factors are SLF proteins (Lai et al., 2002; Sijacic et al., 2004; Kubo et al., 2010). Thus, loss of the pistil-side barrier causes a mating system transition to SC, with potential consequences for the species' evolutionary trajectory (Box 2).

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BOX II: POLLEN-PISTIL INTERACTIONS, MATING SYSTEM TRANSITIONS, AND SPECIATION

Introductory biology textbooks emphasize that speciation (selection, adaptation, and reproductive isolation) is normally a lengthy incremental process requiring millions of years. However, they also acknowledge that, in some cases, speciation can occur almost instantaneously through polyploidization.

We propose that SI plants in the Solanaceae also have a fast-track option for speciation. As described, this type of SI is composed of pistil-side barriers and pollen-side resistance factors. When pistil-side barriers, such as S-RNase, are lost to mutation, then an SI→C mating system transition occurs. This transition can reduce gene flow, since an SC individual is no longer strictly dependent on other individuals for reproduction. It also can facilitate the range expansion of the SC population, thereby further reducing gene flow through geographic separation. Once SI is lost, the other genetic components of the SI system are no longer under selection and additional SI factors, including those involved in pollen-side resistance, can be lost to mutation. If pollen SI factors are lost, the SC population becomes partially (unidirectionally) reproductively isolated from its parent SI population, since its pollen no longer expresses resistance factors. This leads to an additional decrease in gene flow, allowing the SC population to further differentiate from its SI parent population.

This was described recently in *S. habrochaites*, a wild tomato relative (Broz et al., in press). *S. habrochaites* has undergone an SI→C transition at its northern species range margin in the coastal region of central Ecuador. Loss of SI at species range margins is common and is likely selected due to mate or pollinator limitation as species migrate to new environments (Baker, 1955, 1967; Pannell et al., 2015). In this case, the loss of SI is correlated with the loss of S-RNase expression, apparently due to the insertion of a transposable element in the promoter region of the S-RNase gene. These SC populations have undergone additional pollen-side mutations such that their pollen is rejected by styles of nearby SI populations. Furthermore, these SC populations exhibit morphological features associated with a selfing syndrome with smaller, less conspicuous flowers than those of SI populations. Thus, loss-of-function mutations in relatively few pistil- and pollen-expressed SI genes may have driven the formation of a reproductively and morphologically differentiated new lineage, one that may be in the early stages of speciation.

SI in *Antirrhinum* spp. is similar to the Solanaceae system (Xue et al., 1996; Lai et al., 2002; Huang et al., 2006; Chen et al., 2010). SI species in Rosaceae also rely on S-RNases and pollen-expressed F-box proteins; but curiously, species in the Pyreae tribe (e.g. apple [*Malus domestica*] and pear [*Pyrus communis*]) appear to have a nonself recognition system like Solanaceae, while those in *Prunus* (e.g. cherry [*Prunus avium*], apricot [*Prunus armenaica*], and almond [*Prunus dulcis*]) appear to have a self-recognition system (Sassa, 2016).

INTERSPECIFIC REPRODUCTIVE BARRIERS

Numerous adaptations and ecological factors, such as geographical distribution and pollinator specialization, help prevent hybridization between closely related species (Lowry et al., 2008; Widmer et al., 2009; Baack et al., 2015). However, the pistil also is a venue for mate selection at the interspecies level, since it is an arena for postmating, prezygotic barriers that rely on pollen-pistil interactions. This class of interspecific reproductive barriers includes conspecific pollen precedence, the preferential success of conspecific pollen compared with heterospecific pollen (Howard, 1999; Fishman et al., 2008; Lora et al., 2016), and pollen tube guidance and reception. Here, we focus on outright interspecific pollen tube rejection.

SI AND INTERSPECIFIC INCOMPATIBILITY: A COMMON TOOLKIT

Intraspecific and interspecific pollen rejection control mating at opposite ends of the genetic relationship continuum, yet these rejection responses often show similarities. Recent results show that some genes function at both levels and that some mechanisms are similar (see Advances). We view this as a manifestation of the overall function of the pistil as the organ for mate selection.

In a remarkably prescient article, Lewis and Crowe (1958) summarized crosses between many SI species and their SC relatives and emphasized a type of UI where "pollen of the self-compatible species was inhibited in the styles of the self-incompatible species, while no inhibitions occurred in the reciprocal cross." They noted that this so-called SI × SC rule applies to many crosses in the Solanaceae and Scrophulariaceae (McGuire and Rick, 1954) and suggested that it was regulated by the *S*-locus (Lewis and Crowe, 1958). A recent comprehensive study of interspecific compatibility in the tomato clade demonstrated that the SI × SC rule is closely followed at the species level (Baek et al., 2015). In addition to the Solanaceae, UI consistent with the SI × SC rule has been recorded in Lilliaceae (Ascher and Peloquin, 1968), Poaceae (Heslop-Harrison, 1982), Brassicaceae (Sampson, 1962; Hiscock and Dickinson, 1993), and Orchidaceae (Pinheiro et al., 2015). Although the SI × SC rule applies broadly, differences between SI and UI, such as the timing of the response (Fig. 1; Ascher and Peloquin, 1968, Liedl et al., 1996) and the fact that exceptions to the rule can be identified easily, led some to challenge whether the *S*-locus functions at the interspecific level (Hogenboom, 1984; de Nettancourt, 1997, 2001). Recent studies clarify that UI is mechanistically linked to SI in some cases but not in others, and interpretations are complicated by genetic redundancy.

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Genetic and molecular evidence shows that some forms of UI are related to SI. Quantitative trait locus (QTL) studies of tomato clade factors contributing to UI on the pistil side show linkage between the *S*-locus and a major UI QTL (Bernacchi and Tanksley, 1997) and a second that includes the *Solanum HT-A* and *HT-B* genes (Covey et al., 2010). Murfett et al. (1996) provided direct evidence that S-RNase functions in UI, but the results also reveal unexpected complexity. They found that expressing *S-RNase* from SI *N. alata* in SC *Nicotiana plumbaginifolia* pistils caused the rejection of pollen from *Nicotiana tabacum* but not pollen from *N. plumbaginifolia*. However, *N. plumbaginifolia* pollen was rejected if the *S-RNase* transgene was crossed into another background and expressed in conjunction with other pistil-side factors. Thus, *S-RNase* is implicated in rejecting pollen from both *N. tabacum* and *N. plumbaginifolia*, but the mechanisms are different because they show different dependencies on genetic background (Murfett et al., 1996). Other studies showed that HT protein is one of the pistil factors required for *N. plumbaginifolia* pollen rejection (Hancock et al., 2005). Thus, three S-RNase-dependent interspecific pollen rejection mechanisms are distinguished by reliance on modifier genes and pollen-side specificity (Fig. 2). The tomato clade offers additional insights, because its interspecific crossing relationships are better characterized than are *Nicotiana*'s. Expressing *S-RNase* and *HT* genes in SC *S. lycopersicum*, which normally lacks UI barriers, caused the rejection of pollen from all of the red-fruited SC tomato species but not from any green-fruited tomato species (Tovar-Méndez et al., 2014). Taken together, these studies clearly demonstrate that pistil-side SI and UI barriers share genetic factors. We suggest that they rely on a common genetic toolkit and that multiple UI mechanisms use distinct but overlapping sets of factors. This combinatorial aspect may allow the rejection of pollen from a large, variable, and unpredictable number of species with a limited set of genetic factors.

SI and UI also share genetic factors on the pollen side. Chetelat and Deverna (1991) identified three QTL from *S. pennellii* that are required for tomato pollen to overcome an S-RNase-dependent UI barrier. Fine-mapping the *ui6.1* factor identified a *CUL1* gene as a candidate UI factor (Li and Chetelat, 2010). A gain-of-function experiment showed that expressing the *S. pennellii* allele, *SpCUL1*, in conjunction with a chromosome 1 *S. pennellii* introgression, *ui1.1*, containing the *S*-locus was sufficient for pollen compatibility on an S-RNase-expressing pistil-side tester (Li and Chetelat, 2010). A loss-of-function study showed that *CUL1* also functions in SI (Li and Chetelat, 2014). Recently, the *ui1.1* factor was identified as a single *SLF* gene, *SLF-23* (Li and Chetelat, 2015). Thus, SI and UI utilize shared factors for both the pollen- and pistil-side functions and are characterized by a pistil-barrier/pollen-resistance architecture.

OUTSTANDING QUESTIONS

- What are the roles of SI modifier genes? Current models focus on *S*-specificity determinants. Elucidating the roles of HT, 120K, and NaStEP in the S-RNase-based system and ARC1 and exo70A1 in the Brassicaceae system will reveal much about the physiology of SI.
- How does S-RNase/SLF interaction lead to nonself S-RNase recognition? What is the temporal sequence of events? Why does it take hours or days to stop growth of incompatible pollen tubes?
- What are the molecular mechanisms of interspecific pollen rejection? What is the relationship between mating system transitions and speciation? How do the biogeographic and community contexts of a species interact with the genetic and molecular architecture of mate recognition as it adapts and evolves?

DIFFERENCES BETWEEN SI AND UI: ADDITIONAL UI MECHANISMS

Although there is overlap between SI and UI, they are not identical. For instance, there are many exceptions to the SI × SC rule (e.g. SC × SC incompatibilities; Baek et al., 2015), and there are physiological and morphological differences between SI and UI. For example, pollen tube staining of pollinated pistils often shows that interspecific pollen tube rejection occurs closer to the stigma than SI pollen rejection (Fig. 1; Lewis and Crowe, 1958; Martin, 1964; Ascher and Peloquin, 1968; Liedl et al., 1996; Covey et al., 2010). In addition, ultrastructural studies comparing SI and UI in *Solanum peruvianum* self-pollinations versus cross-pollinations with *S. lycopersicum* show that the outer (noncallose, largely pectic) pollen tube cell wall appears to thicken during SI rejection and to be degraded during UI rejection (de Nettancourt et al., 1973, 1974).

Crucially, the level of specificity in SI and UI differs dramatically. SI is exquisitely specific and only causes the rejection of pollen with specific *S*-haplotypes, while UI interspecific pollen rejection causes the rejection of pollen from entire species or clades (Murfett et al., 1996; Beecher et al., 2001; Tovar-Méndez et al., 2014). Lewis and Crowe (1958) noted that pistils with any functional *S*-locus could inhibit interspecific SC pollen, and they referred to this as unitary action. Modern gain-of-function

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studies (Murfett et al., 1996; Beecher et al., 2001; Tovar-Méndez et al., 2014) confirm this large difference in specificity. The exact role (or roles) of S-RNase in UI could differ from its role in SI. In UI, for example, it is possible that S-RNases are not involved in pollen recognition per se but rather function downstream in signaling or inhibitory components of the pollen-rejection pathway. This type of system - separate recognition systems coupled to a common downstream pathway - has been proposed in Poaceae (Heslop-Harrison, 1982) and Brassicaceae (Kitashiba and Nasrallah, 2014). In fact, the rapid interspecific pollen tube rejection seen in UI (Fig. 1) may reflect a rapid deployment of S-RNase toxicity based on an alternative recognition system, or it is possible that S-RNase compartmentalization and degradation make different contributions to pollen resistance in SI and interspecific contexts.

Functional studies confirm that interspecific pollen rejection is more complex than SI in the sense that multiple UI mechanisms contribute to compatibility/incompatibility and, in addition, that redundancy is common. Redundancy is apparent in systems where gain-of-function studies show that pollen that is susceptible to S-RNase-dependent rejection (such as the *Nicotiana* and tomato studies described earlier; Murfett et al., 1996; Beecher et al., 2001; Tovar-Méndez et al., 2014) can also be rejected by SC accessions that entirely lack S-RNase (Martin, 1961; Murfett et al., 1996; Covey et al., 2010; Chalivendra et al., 2013; Tovar-Méndez et al., 2014; Baek et al., 2015; Broz et al., 2017). Redundancy complicates analysis, but the mechanisms can be elucidated through a combination of gain- and loss-of-function studies. It is difficult to understand the maintenance of multiple redundant pollen rejection systems. Laboratory experiments necessarily test a limited number of species on both the pistil and pollen sides. However, in natural contexts, outcrossing species' pistils are challenged by pollen from many species (Ashman and Arceo-Gómez, 2013); therefore, they may deploy a variety of interspecific pollination barriers that appear to be redundant under laboratory conditions. We suggest that, overall, incompatibility between species can be understood as arising from the additive effects of the complement of pistil barriers and pollen resistance factors expressed in each species. In any case, elucidating these S-RNase-independent mechanisms for interspecific pollen rejection will provide new insights.

The nature and diversity of S-RNase-independent UI mechanisms are just beginning to be understood. Eberle et al. (2012, 2013) used an elegant semi in vivo assay to investigate SC × SC incompatibility between *N. tabacum* and relatives, such as *Nicotiana repanda*. They used genetic ablation to create hollow *N. tabacum* pistils with little or no transmitting tract and showed that the ability to reject pollen from *N. repanda* also was lost. However, when *N. tabacum* pistil extracts were introduced, pollen rejection was restored. Fractionation experiments suggested that the *N. tabacum* PELPIII (Pistil Extensin-Like Protein III) protein is sufficient to cause rejection in this assay (Fig. 2; Eberle et al., 2013). To our knowledge, this is the first example of a specific protein associated with S-RNase-independent pollen rejection.

There are also several examples of S-RNase-independent UI in the tomato clade. Loss of S-RNase expression or enzymatic activity has been documented in at least three SC accessions of otherwise SI species (Kondo et al., 2002; Covey et al., 2010) that nevertheless reject pollen from SC species, such as *S. lycopersicum* (Liedl et al., 1996; Baek et al., 2015). Chalivendra et al. (2013) showed that UI pollen rejection in one of these accessions (SC *S. pennellii* LA0716) is developmentally regulated in a manner similar to SI: interspecific pollen is accepted by very young pistils but rejected on mature pistils. This observation is important because it suggests that UI barriers are layered over an otherwise compatible state of the pistil and that UI genes and SI genes likely show similar developmental profiles. A recent study by Pease et al. (2016) exploited a transcriptomic approach to identify candidate UI genes. Among the top 20 genes showing high expression in UI-competent styles, cell wall/cell wall modification (five in the top 20) genes were prominent. One especially intriguing *S. pennellii* gene encodes a pectin methylesterase inhibitor (PMEI) that appears to be absent in the *S. lycopersicum* genome. The methylation state of pectin is critical to normal pollen tube growth; demethylated pectins form a rigid gel in the shaft of the pollen tube to allow the growth of pollen tubes through the transmitting tissue of the style, whereas PMEIs are localized specifically to the expandable and flexible tube apex, a structure that can respond to signaling and change directional growth (Bosch et al., 2005; Bosch and Hepler, 2005, 2006; Röckel et al., 2008; Hepler et al., 2013). Mutations in an Arabidopsis PMEI gene, *VGD1*, result in aberrant pollen tube growth and instability (Jiang et al., 2005), and exogenously adding maize (*Zea mays*) ZmPMEI1 protein causes the bursting of pollen tubes growing in vitro (Woriedh et al., 2013). Thus, it is plausible that the *S. pennellii* LA0716 PMEI gene could contribute to UI.

CONCLUSION

Genes that control mate selection control gene flow. The angiosperm pistil is the organ where many mate selection genes act. While it would be unwise to overemphasize one slice of the life cycle, these genes surely bear special importance in the generation of angiosperm diversity. The prevalence of SI systems and the persistence of SI lineages (Goldberg et al., 2010; Goldberg and Igić, 2012) are good evidence that genetic mechanisms controlling mating are adaptive.

Thirty years of research have identified genes that control the specificity of SI as well as some of the underlying physiological mechanisms. Yet to be determined are the biochemical interactions that define *S*-specificity and the roles of modifier genes that do not contribute to *S*-specificity per se (see Outstanding Questions). SI research has advanced, but it is only one

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example of a pollen-pistil interaction that controls mating. At present, we know that SI and interspecific pollination mechanisms share some genes and mechanisms, and it is now possible to clearly determine when they are similar to SI and when they are not. It also should be possible to better understand how the pollen-pistil genetic toolkit is applied to control mating in a broader sense.

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