LOSS OF SELF-INCOMPATIBILITY AND ITS EVOLUTIONARY CONSEQUENCES

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We review and analyze the available literature on the frequency and distribution of self-incompatibility (SI) among angiosperms and find that SI is reported in more than 100 families and occurs in an estimated 39% of species. SI frequently has been lost but rarely has been gained during angiosperm diversification, and there is no evidence that any particular system of SI, once lost, has been regained. Irreversible loss of SI systems is thought to occur because transitions to self-compatibility (SC) are accompanied by collapse of variation at the *S*-locus and by accumulation of loss-of-function mutations at multiple loci involved in the incompatibility response. The asymmetry in transitions implies either that SI is declining in frequency or that it provides a macroevolutionary advantage. We present a model in which the loss of SI is irreversible and species can be SI, SC but outcrossing, or predominantly selfing. Increased diversification rates of SI relative to SC taxa are required to maintain SI at equilibrium, while transition rates between states, together with state-specific diversification rates, govern the frequency distribution of breeding-system states. We review empirical studies about the causes and consequences of the loss of SI, paying particular attention to the model systems *Arabidopsis* and *Solanum* sect. *Lycopersicon*. In both groups, losses of SI have been recent and were accompanied by loss of most or all of the functional variation at the *S*-locus. Multiple loss-of-function mutations are commonly found. Some evidence indicates that mutations causing SC strongly increase the selfing rate and that SC species have lower genetic diversity than their SI relatives, perhaps causing an increase in the extinction rate.

Keywords: self-incompatibility, ancestral state reconstruction, irreversibility, mating system, outcrossing, selfing, *S*-locus.

The evolutionary pathway from obligate outcrossing based upon self-incompatibility
to predominant self-fertilization has probably been followed by more different lines
of evolution in flowering plants than has any other. (Stebbins 1974, p. 51)

Introduction

The vast majority of flowering plants are simultaneous hermaphrodites (Yampolsky and Yampolsky 1922). Despite many potential advantages of self-fertilization (Darwin 1876; Fisher 1941; Baker 1955, 1967; Schoen et al. 1996; Schoen and Busch 2007), most angiosperms possess some mechanism that greatly reduces or prevents it. Current estimates indicate that outcrossing is enforced by self-incompatibility (SI) or dioecy in approximately half of all angiosperm species (Igic and Kohn 2006). Here we are primarily concerned with SI, broadly defined as any postpollination prezygotic mechanism that prevents self-fertilization. We focus on the well-described homomorphic SI mechanisms in which the molecular bases for self-pollen recognition and rejection are at least partially understood (Takayama and Isogai 2005; McClure 2006), the history of a particular mechanism can be traced (Igic and Kohn 2001; Steinbachs and Holsinger 2002; Castric and Vekemans 2004; Igic et al. 2006), and the causes and consequences of its loss can be documented.

We first review the phylogenetic distribution of SI systems among angiosperms. SI systems have arisen many times, though one form of homomorphic SI, the RNase-based gametophytic system found in the Solanaceae, Plantaginaceae, and Rosaceae, is the ancestral state for the majority of dicots (Igic and Kohn 2001; Steinbachs and Holsinger 2002). We show that, while SI clearly has multiple origins, losses of SI vastly outnumber gains. Reconstructing ancestral states and estimating rates of transition between SI and self-compatibility (SC) states has been a goal of many studies (e.g., Weller et al. 1995; Barrett et al. 1996; Igic et al. 2004; Mast et al. 2006; Ferrer and Good-Avila 2007). Here we caution against such practices using the currently available tools. Phylogenetic methods for estimating rates of gain and loss of characters based on the states of extant taxa (e.g., Pagel 1994, 1997) rely on an assumption that differences in character state have no effect on lineage diversification rates (Igic et al. 2006; Maddison 2006). If this assumption is violated, as we show is likely for SI, estimates of transition rates and ancestral states will often be misleading, as has been demonstrated for the Solanaceae (Igic et al. 2004, 2006).

We also review the current state of knowledge about the frequency of SI in angiosperms. Because losses of SI appear far more common than gains, the high frequency of SI among

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[SI]	TRIMENIACEAE
[SI]	LUZURIAGACEAE
[GSI]	LILIACEAE
[GSI]	IRIDACEAE
[GSI/Het]	AMARYLLIDACEAE
[SI]	AGAVACEAE
[SI/Het]	TECOPHILAEACEAE
[GSI]	ORCHIDACEAE
[GSI]	**POACEAE**
[GSI]	BROMELIACEAE
[SI]	HELICONIACEAE
[GSI]	ZINGIBERACEAE
[Het]	PONTEDERIACEAE
[SI]	COMMELINACEAE
[SI]	CYCLANTHACEAE
[GSI]	ARACEAE
[SI]	ANNONACEAE
[SI]	WINTERACEAE
[SI]	SAURURACEAE
[GSI]	**PAPAVERACEAE**
[SI]	FUMARIACEAE
[SI]	BERBERIDACEAE
[GSI]	RANUNCULACEAE
[SI]	PROTEACEAE
[GSI*]	**ROSACEAE**
[SI]	RHAMNACEAE
[GSI]	FAGACEAE
[SSI]	BETULACEAE
[GSI/Het]	FABACEAE
[SI]	CELASTRACEAE
[Het]	OXALIDACEAE
[Het]	CONNARACEAE
[Het]	ERYTHROXYLACEAE
[GSI]	PASSIFLORACEAE
[Het]	TURNERACEAE
[SI]	CHRYSOBALANACEAE
[Het]	LINACEAE
[SI/Het]	CLUSIACEAE
[SI]	MALPIGHIACEAE
[SI]	EUPHORBIACEAE
[SI]	COCHLOSPERMACEAE
[SSI/Het]	MALVACEAE
[SI]	DIPTEROCARPACEAE
[SI]	CISTACEAE
[SI]	RUTACEAE
[SI]	MELIACEAE
[SI]	SAPINDACEAE
[SI]	ANACARDIACEAE
[SSI]	**BRASSICACEAE**
[GSI]	RESEDACEAE
[GSI]	TROPAEOLACEAE
[SI]	MYRTACEAE
[SI]	MELASTOMATACEAE
[GSI]	ONAGRACEAE
[SI/Het]	LYTHRACEAE
[SSI]	GERANIACEAE
[SI]	STAPHYLEACEAE
[GSI]	HAMAMELIDACEAE
[GSI/Het]	SAXIFRAGACEAE
[SI]	CRASSULACEAE
[GSI]	PAEONIACEAE
[SSI]	CARYOPHYLLACEAE
[GSI]	AMARANTHACEAE
[SSI]	NYCTAGINACEAE
[GSI]	CACTACEAE
[Het]	PLUMBAGINACEAE
[SI/Het]	POLYGONACEAE
[SI]	DROSERACEAE
[Het]	OLACACEAE
[Het]	SANTALACEAE
[GSI]	CORNACEAE
[GSI]	ERICACEAE
[SI]	THEACEAE
[SI]	LECYTHIDACEAE
[SI]	SAPOTACEAE
[GSI]	MYRSINACEAE
[Het]	PRIMULACEAE
[SSI/GSI]	POLEMONIACEAE
[SI]	FOUQUIERIACEAE
[GSI*]	**SOLANACEAE**
[SSI]	CONVOLVULACEAE
[SI/Het]	BORAGINACEAE
[SI]	GESNERIACEAE
[GSI*]	**PLANTAGINACEAE**
[GSI]	SCROPHULARIACEAE
[SI]	LENTIBULARIACEAE
[GSI]	BIGNONIACEAE
[GSI/Het]	ACANTHACEAE
[SI]	OROBANCHACEAE
[GSI/Het]	OLEACEAE
[GSI/Het]	RUBIACEAE
[GSI]	APOCYNACEAE
[SI/Het]	LOGANIACEAE
[Het]	GENTIANACEAE
[GSI]	**CAMPANULACEAE**
[Het]	MENYANTHACEAE
[GSI]	GOODENIACEAE
[SSI]	ASTERACEAE
[SI]	ARGOPHYLLACEAE
[SSI]	CAPRIFOLIACEAE

angiosperm species suggests that SI lineages may often be associated with higher net diversification rates (Igic et al. 2004). We expand an earlier model (Igic et al. 2004) to involve transitions between three states: SI, SC with predominant outcrossing, or predominant selfing. This model can lead to stable maintenance of SI only if it is associated with increased diversification. The balance of transition rates and state-specific diversification rates determines the relative frequency distribution of states.

Once lost, any system of homomorphic SI is difficult to regain, for at least two reasons. First, polymorphism at the S-locus is expected to collapse over time when rendered selectively neutral by the transition to SC. Preexisting polymorphism is needed for the evolution of these systems (Charlesworth and Charlesworth 1979). Second, all well-characterized homomorphic SI systems rely on the coordinated functions of several genes, both linked and unlinked to the S-locus (Takayama and Isogai 2005; McClure 2006). Additional loss-of-function mutations are expected to accumulate after the transition to SC, increasing the difficulty of reversal. We assess the validity of these predictions by reviewing what is known about S-locus polymorphism and the mutations responsible for SC in model systems where SC has recently evolved, the genus Arabidopsis and Solanum sect. Lycopersicon (the wild and cultivated tomatoes). Motivated by the observation that SI is likely to be associated with higher net diversification rates, we also examine the consequences of the transition to SC on the mating system, genomewide genetic variation, and the evolutionary potential of lineages.

The Distribution of SI

At least 100 flowering plant families reportedly contain SI species (fig. 1). This is probably a conservative estimate because the appropriate studies (see Charlesworth 1985) to demonstrate SI are rarely performed or are infrequently reported. The expression of SI cannot be described monolithically because the underlying mechanisms differ widely among groups. Textbook classifications are most often based on the presence or absence of variation in morphology between mutually compatible flowers (heteromorphic or homomorphic) and the genetic mode of action (gametophytic or sporophytic). Less frequently, the site of expression (stigma, style, or ovary) and the number of loci involved in determining the self-recognition phenotype are also reported (de Nettancourt 1977). Presently, species in at least 25 plant families are known to express heteromorphic SI (fig. 1; Ganders 1979; Gibbs 1990; Barrett 1992; Steinbachs and Holsinger 2002), and at least 94 families express some manner of homomorphic SI (Gibbs 1990; Weller

et al. 1995; Steinbachs and Holsinger 2002; B. Igic, unpublished data). Within groups with homomorphic SI, the sporophytic genetic mode of control (SSI), in which the female evaluates the diploid paternal genome of each pollen grain, is found in 10 families. The gametophytic mode of SI (GSI), in which the haploid genome of a pollen grain is evaluated by the female, occurs in 36 families. In 47 families, homomorphic SI is reported, but without sufficient information to ascertain the genetic mode of action. Although heteromorphic and homomorphic SI coexist within 12 families, the classification of homomorphic SI within families is apparently invariant, with the exception of Polemoniaceae (Levin 1993; Goodwillie 1997). Invariance at the family level is often the basis for the assumption that SI is homologous within relatively old monophyletic groups (i.e., families), an assumption that holds true for the few families in which the molecular basis of incompatibility is known and multiple genera have been studied (Rosaceae, Solanaceae, Plantaginaceae, and Brassicaceae; reviewed in Castric and Vekemans 2004). Although classifications such as SSI and GSI can be didactically helpful, families sharing the same form of SI cannot be assumed to do so by homology (Gibbs 1986).

However, in one case, that of homomorphic, RNase/F-box-controlled (McClure 2006) GSI found in three distantly related eudicot families (Rosaceae, Solanaceae, and Plantaginaceae), the shared molecular basis of incompatibility, coupled with the structural and phylogenetic relationships of some of the underlying genes, strongly implies homology (Igic and Kohn 2001; Steinbachs and Holsinger 2002; Ushijima et al. 2003; Qiao et al. 2004; Sijacic et al. 2004). This finding rejects the view (e.g., Weller et al. 1995) that the ancestor of most eudicots (ca. 90–100 million years ago) was SC. Instead, it implies that the common ancestor of ca. 75% of dicots was GSI and that SSI arose independently at least 10 times within higher eudicots (fig. 1; Igic and Kohn 2001; Steinbachs and Holsinger 2002). Furthermore, the phylogenetically scattered occurrence of the families that possess species with heteromorphic SI together with the shared ancestry of GSI in the Rosid and Asterid lineages both strongly suggest that heteromorphic SI evolved at least 22 times in flowering plants (fig. 1; see also Barrett 1992). In addition, homomorphic GSI evolved independently at least four times. Nonhomologous GSI systems, employing different molecular mechanisms, are known to operate outside the Rosid/Asterid clade in the Papaveraceae (Foote et al. 1994) and Poaceae (Baumann et al. 2000). Within the Asterids, the Campanulaceae are now also suspected to possess a form of GSI that does not rely on the RNase/F-box mechanism (S. Good-Avila and A. Stephenson, personal communication). Overall, some form of SI evolved independently at least 35 times in angiosperms.

Fig. 1 Distribution of self-incompatibility (SI) in 105 families of angiosperms. The hypothesis of relationships among families derives from Davies et al. (2004), as implemented in *Phylomatic* (Webb and Donoghue 2005). The status of families was collected from literature searches by Gibbs (1990), Weller et al. (1995), Steinbachs and Holsinger (2002), and B. Igic (unpublished data). SI system designations are encoded as follows: [GSI] = homomorphic gametophytic SI, [SSI] = homomorphic sporophytic SI, [SI] = homomorphic SI, uncharacterized mode of action, and [Het] = heteromorphic SI. Any combination indicates the presence of multiple systems within the designated family. The extent of evidence for SI is variable. Note that Rosaceae, Solanaceae, and Plantaginaceae share the homologous RNase-based GSI mechanism (each is marked by an asterisk, as is the ancestral node). Families with SI systems whose genetic basis is known and molecular basis is at least partially characterized are in bold. Although the molecular basis for Campanulaceae is unknown, it appears not to be controlled by the RNase-based system.

SI to SC Transitions

Although new forms of SI have evolved many times, on simple mechanistic grounds, mutations are far more likely to cause the loss of SI than its gain (reviewed in Stone 2002). The characterized homomorphic SI systems use distinct genes for pollen and pistil recognition phenotypes, and many accessory genes are also necessary for the proper function of SI. Thus loss-of-function mutations at many loci can lead to the breakdown of SI. The loss of SI releases other participating loci from selective pressure, making further degradation of the system likely. By comparison to the loss of SI, the buildup of the requisite pathways and variability for self-recognition and rejection appears considerably more difficult. This makes the loss of SI a likely example of an evolutionary transition that is extremely difficult to reverse (Marshall et al. 1994; Igic et al. 2006). Without exception, large-scale studies performed in SI species find individuals or populations that recently became SC, and many studies show that the breakdown occurred more than once within species (e.g., Rick and Chetelat 1991; Tsukamoto et al. 1999, 2003a, 2003b; Sherman-Broyles et al. 2007; Shimizu et al., forthcoming). By comparison, a convincing case for the recent development of one or more forms of SI within any species would be far more unusual.

In addition to these verbal arguments for why loss of SI should be common and reverse transitions extremely difficult, there is strong genetic evidence that, at the level of at least one large family, the loss of SI has been frequent and irreversible. In the Solanaceae, a family of some 2600 species, of which ca. 40% are SI (Whalen and Anderson 1981; Igic et al. 2006), widespread shared ancestral polymorphism at the S-locus implies an uninterrupted ancestry of SI for all species from which S-alleles have been sampled (for a more complete discussion, see Igic et al. 2006). When inference from shared ancestral polymorphism is used to inform reconstructions of the rates of gain and loss of SI, the loss of SI is found to be effectively irreversible (rate of gain = 0 cannot be rejected; Igic et al. 2006). However, in the absence of information from shared ancestral polymorphism, the opposite and incorrect conclusion is reached, that transitions in both directions have occurred.

Why do reconstructions of transition rates fail when applied to the question of SI when only the character states of extant taxa are used? Recent work suggests that current reconstruction methods (Sanderson 1993; Maddison 1994; Pagel 1994; Schluter et al. 1997) can fail if net diversification rates vary with character state (Igic et al. 2006; Maddison 2006). These methods implicitly assume that the character states being reconstructed do not influence rates of diversification (Igic et al. 2006; Maddison 2006). Simulations show that violation of the assumption of equal diversification rates leads to reconstructions that falsely reject the character transition rates used to simulate the data (Maddison 2006). Reconstructed transition rates overestimate the rate of transition to the character state that confers higher diversification. We show below that there are strong reasons to believe that net diversification rates are higher in SI than in SC taxa. If this is true, reconstructions that ignore differences in diversification rates (e.g., Ferrer and Good-Avila 2007) will falsely inflate the rate of transition from SC to SI. Similar caution applies to reconstructions involving other character state transitions, when alternative states may cause differential net diversification, including the evolution of selfing versus outcrossing, specialists versus generalists, and sexuality versus asexuality, among many others.

Although the number of gains of SI in angiosperms may appear surprisingly high, losses vastly outnumber gains. For instance, SI has been lost a minimum of 60 times in the Solanaceae (Igic et al. 2006) and eight to nine times in the tomatoes (Solanum sect. Lycopersicon) alone (fig. 2). Like the Solanaceae, most families appear to have a single homologous mechanism of SI (but see Goodwillie 1997). Within-family homology has also been verified in the Brassicaceae, Plantaginaceae, and Rosaceae, where the molecular basis of incompatibility is known (reviewed in Castric and Vekemans 2004). Each of these families possesses a large and phylogenetically widespread number of SC species (Heilbuth 2000). Consequently, we expect that the pattern of frequent losses of SI is common.

The Frequency of SI

For several reasons, our knowledge of the frequency of SI among angiosperm species is inadequate. The estimate of the frequency of SI (ca. 40%; table 1) comes from 27 published surveys of breeding systems in New World plant communities. This is somewhat lower than an earlier estimate (ca. 50%) by Darlington and Mather (1949), though it is far higher than the representation of SI species in the studies of the distribution of outcrossing rates (Igic and Kohn 2006). In community surveys, sampling schemes and experimental methods differ, with some authors exclusively choosing woody or herbaceous species and often employing different criteria for the classification of SI and SC species. We recoded the data using a consistent cutoff for the value of the index of SI (ISI, the relative success of selfed vs. outcrossed seed or fruit set; Bawa 1974) of 0.2. Changing this value to 0.1 does not substantially change the trends reported below, nor does the application of
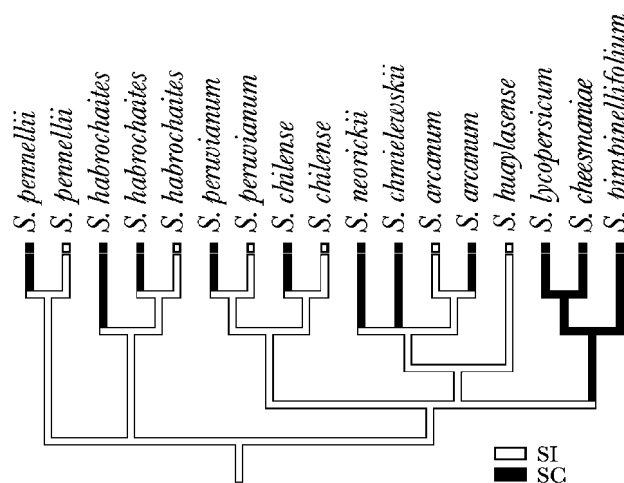
Fig. 2 Evolution of breeding systems in *Solanum* sect. *Lycopersicon*. The phylogenetic hypothesis is modified from Spooner et al. (2005). Data on breeding-system status are derived from Kondo et al. (2002a), Peralta and Spooner (2001), Rick and Chetelat (1991), and B. Igic (unpublished data). SI = self-incompatible; SC = self-compatible.

Table 1

Frequency of Self-Incompatibility (SI) in Different Plant Communities

Flora	n	SI (%)	Habit	Study
Temperate:				
Canadian forest herbs	12	33.3	Herbaceous	Barrett and Helenurm 1987
Canadian salt marsh	17	23.5	Herbaceous	Pojar 1974
Canadian bog	28	25.0	Herbaceous	Pojar 1974
Canadian subalpine meadow	37	40.5	Herbaceous	Pojar 1974
New England shrubs	12	8.3	Woody	Rathcke 1988
North Carolina forest wildflowers	11	27.3	Herbaceous	Motten 1986
Arizona desert	14	92.9	Woody	Neff et al. 1977
Argentine deserts	12	58.3	Woody	Neff et al. 1977
Patagonian alpine flora	124	28.0	Herbaceous[a]	Arroyo and Squeo 1990
Chilean temperate dry forest	37	34.4	Mixed	Arroyo and Uslar 1993
Chilean valvidian forest	39	30.0	Mixed	Riveros et al. 1996
Argentine chaco forest	15	60.0	Mixed	Aizen and Feinsinger 1994
Argentine chaco forest	32	43.8	Mixed	Morales and Galetto 2003
Argentine chaco understory	7	85.7	Woody	Bianchi et al. 2000
Tropical:				
Mexican deciduous forest	33	65.4	Mixed	Bullock 1985
Costa Rican dry forest	34	61.7	Woody	Bawa 1974
Costa Rican lowland forest	64	43.9	Mixed	Kress and Beach 1994
Brazilian Caatinga, semiarid	36	56.7	Mixed	Machado et al. 2006
Brazilian savanna, near Brasilia	30	70.6	Woody	Oliveira and Gibbs 2000
Venezuelan tropical dry forest	49	50.7	Mixed	Jaimes and Ramírez 1999
Venezuelan palm swamp	25	19.4	Mixed	Ramírez and Brito 1990
Venezuelan cloud forest	25	37.0	Mixed	Sobrevila and Arroyo 1982
Island:				
Chiloé Island, Chile	20	45.0	Woody	Smith-Ramírez et al. 2005
Galapagos Islands, Ecuador	51	1.9	Mixed	McMullen 1990
Juan Fernandez Islands, Chile	22	13.6	Mixed	Anderson et al. 2001
Jamaican montane forest	8	9.9	Woody	Tanner 1982
New Zealand	47	18.2	Mixed	Newstrom and Robertson 2005

Note. Studies are considered to be tropical if they were performed between the Tropics of Cancer and Capricorn, and if not, they were considered temperate. Of the island studies, only that of McMullen (1990) is tropical by this criterion.

[a] Two woody species were examined in the study but excluded in our analyses.

sample size weighting, which lowers the frequency estimate to 36.7%.

At least three trends can be gleaned from the published surveys of the frequency of SI in natural plant communities (table 1; fig. 3). First, species on oceanic islands are far less likely to be SI (Wilcoxon test $W = 94$, $P < 0.02$), providing yet another line of evidence for "Baker's Rule," the predicted association of isolated and peripheral habitats with SC (Baker 1955, 1967). This holds true even though the sample size for the islands (five surveys) is small and despite the fact that one island group, the Chiloé Islands, was connected with the mainland for much of the Pleistocene (Villagrán 1988). Second, studies of woody taxa generally find a higher frequency of SI than those focused on herbaceous taxa ($W = 6$, $P = 0.065$; island studies excluded). Because of the possibility of increased rates of somatic mutation, Scofield and Schultz (2006) suggest that woody species may be unable to purge genetic load, perhaps selecting for the maintenance of SI. The trend for lower frequency of SI in herbaceous taxa, however, is highly confounded with the third trend; SI is potentially more common in tropical than in temperate species (fig. 3; $F = 3.253$, $P = 0.086$). This possibility was recognized by Dobzhansky (1950), who posited that repeated glacial advances and successive continental changes between arid and pluvial climates ensured more recent

colonization of temperate areas. Consequently, the higher latitudes could harbor a greater proportion of species with derived opportunistic traits (such as selfing or SC, e.g.), which may provide a temporary advantage at the price of future adaptive potential. Dobzhansky (1950) clearly viewed the sacrifice of outcrossing for reproductive assurance by selfing as a doomed evolutionary gambit that is more prevalent in the temperate zones: "Although some plant species native in the tropics have also become stranded in these evolutionary blind alleys, the incidence of such species is higher in and near the regions which were glaciated" (p. 219). He proposed that there should be a latitudinal gradient in the proportion of outcrossing plants. While his 1950 paper contains few specifics, he is rarely credited with the ideas that appear to have anticipated or coexisted with those of Baker (1955) and Stebbins (1957, 1974), positing that recently colonized areas would contain more selfers, that transitions from SI to SC are common, and that the transition from outcrossing to selfing is an evolutionary dead end.

Several potentially confounded variables, such as the proportions of woody/herbaceous, tropical/temperate, and island/mainland species, are generally unknown. Consequently, our estimate of the frequency of SI, while clearly showing that it is common, will certainly be revised as more information becomes available. Continental- or global-scale databases on species
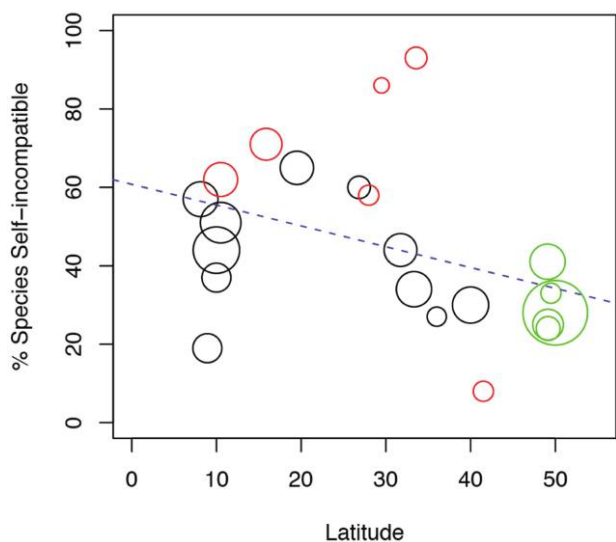
Fig. 3 Relationship between the frequency of self-incompatibility in community surveys and latitude. Circled areas are scaled indicators of sample sizes (see table 1), color coded to reflect the primary makeup of the surveyed floras: green = herbaceous, red = woody, black = mixed.

growth form and range will be helpful in determining these relative proportions. The present data on the frequency of SI in nature are far from definitive, and additional community surveys are badly needed.

The Model

We have shown above that SI is phylogenetically widespread, quite frequent among species, and its loss is irreversible except in the relatively rare instances when novel SI systems arise. Common and essentially irreversible loss of SI implies that SI is either declining in frequency or that it confers a macroevolutionary advantage. We previously presented a deterministic equilibrium model for the stable coexistence of SI and SC taxa (Igic et al. 2004). Here, we expand that model to include two distinct SC groups, those that are predominant outcrossers and those that are selfers. Our model is similar to Nunney's (1989) model for the evolutionary maintenance of sex, and it has similarities with the early portions of the model of Schoen and Busch (2007) for the evolution of self-fertilization in a metapopulation. It assumes exponential growth in the number of species and only allows for irreversible transitions from SI to SC outcrossing and from SC outcrossing to SC selfing, a useful heuristic approach. We do not mean to imply that SC outcrossing taxa inevitably become highly selfing, but as they cannot return to SI, they and their descendants can either remain SC but outcrossing or evolve to become highly selfing. We assume the highly selfing state to be absorbing because, if SI confers an increase in diversification rate relative to both SC groups, it is reasonable that it does so because selfing is often an evolutionary dead end. The advantage of this three-state model over our previous model (Igic et al. 2004) is that it allows for SC taxa that vary in outcrossing rate and that it can also predict the frequency of various mating system states.

Let N_I, N_O, and N_S be the numbers of species with the three character states SI, SC outcrossing, and SC selfing, respectively. Given that each state has an associated exponential rate parameter (r_I, r_O, and r_S) defined for each state as the speciation rate minus the extinction rate, and the only appreciable transitions occur from SI to SC outcrossing (l_I) and from SC outcrossing to SC selfing (l_O), the change in expected numbers in each group over time can be expressed as

$$\frac{dN_I}{dt} = (r_I - l_I)N_I,$$
$$\frac{dN_O}{dt} = l_I N_I + (r_O - l_O)N_O,$$
$$\frac{dN_S}{dt} = l_O N_O + r_S N_S.$$

If the starting condition is a single SI lineage, the results depend on which of the following net diversification rates is largest: r_S, $r_O - l_O$, or $r_I - l_I$. Assuming that at least one of these quantities is positive, if r_S is largest, then both N_I and N_O approach zero frequency, while only N_S increases at the rate r_S. If $r_O - l_O$ is the largest, then N_I asymptotically approaches zero frequency, while N_O and N_S increase at the rate $r_O - l_O$, attaining the ratios

$$(N_O, N_S) \; \propto \; \left(1, \frac{l_O}{r_O - l_O - r_S}\right).$$

Finally, if $r_I - l_I$ is the largest, then all three types asymptotically increase in numbers at the rate $r_I - l_I$, attaining the ratios

$$(N_I, N_O, N_S) \; \propto$$
$$\left[1, \frac{l_I}{r_I - l_I - r_O + l_O}, \frac{l_O l_I}{(r_I - l_I - r_S)(r_I - l_I - r_O + l_O)}\right]. \quad (1)$$

SI systems have persisted for many millions of years (Igic and Kohn 2001; Igic et al. 2006), and transition rates to SI are extremely small in comparison to transitions from SI to SC. Consequently, the most likely reason for the abundance of SI species in nature is that the conditions for equation (1) are met. Only under this condition are appreciable frequencies of SI species maintained at equilibrium. This condition requires that the net diversification rate (speciation − extinction − loss) of SI lineages exceed that of SC outcrossing or selfing lineages.

Despite Stebbins's (1957) conjecture that selfing is an evolutionary dead end, lineage-specific net diversification rates have rarely been advanced as factors governing the current distribution of plant mating systems (Igic and Kohn 2006). While recent microevolutionary models of plant mating systems examine the stability of mixed mating (Goodwillie et al. 2005 and references therein; Porcher and Lande 2005a), even those that predict only predominant outcrossing and selfing as stable states (Lande and Schemske 1985) do not predict the relative frequencies of selfers and outcrossers. This simple model makes explicit the relation between state-specific diversification and transition rates, and the equilibrium proportions of mating system states. Our model can be elaborated to capture additional complexities of mating system evolution and to more

closely approximate the true distribution of mating systems when known.

The Loss of SI

While phylogenetic analyses and macroevolutionary models are important tools for understanding broad evolutionary patterns, microevolutionary studies can focus on the causes and consequences of particular mating system shifts. For single-locus multiple-allele SI systems, several predictions can be made about the near-term effects of the loss of SI. First, if mutations to SC substantially reduce the outcrossing rate, they are unlikely to be selectively neutral. Within a population, the fate of SC mutations will be determined principally by the magnitudes of inbreeding depression, pollen discounting, and the power of selection favoring reproductive assurance (reviewed in Barrett 2002; Schoen and Busch 2007). Drift is probably unimportant except for cases of long-distance dispersal, and, even there, selection will play a role by favoring individuals capable of uniparental reproduction when plant or pollinator abundance is low. Second, many SI species display multiple flowers simultaneously, both within and among inflorescences. The opportunity for geitonogamy suggests that a mutation causing loss of SI will often substantially reduce the primary outcrossing rate, even in the absence of subsequent changes in floral features such as size that might further lead to the evolution of a selfing syndrome. Third, transition to SC renders the extraordinary number of S-alleles in a population selectively neutral. After transition to SC, alleles at the S-locus will no longer be protected by negative frequency-dependent selection. Fixation on a single S-allele is expected to occur in $4N_e$ generations (N_e = effective population size; Hudson 1990), on average, if the mutation causing SC is unlinked to the S-locus and more rapidly if loss of SI is caused by a selective sweep of a nonfunctional S-allele. Following loss of SI, particularly where the mating system proceeds toward predominant selfing, genomic changes in the levels and distribution of genetic variation associated with the outcrossing to selfing transition may ensue (Glemin et al. 2006; Wright et al. 2007).

The best-studied system for investigating a particular transition from SI to SC is the genus *Arabidopsis*, where the SI sister species *A. lyrata* and *A. halleri* are the closest relatives of the highly selfing *A. thaliana* (Koch et al. 2000, 2001). Divergence of the lineage leading to SC *A. thaliana* from its SI sister group is thought to have occurred ca. 5 million years ago (Koch et al. 2000, 2001; Ramos-Onsins et al. 2004). However, when the mating system changed relative to this divergence has been an open question. SI *Arabidopsis* species have the SSI system found in other Brassicaceae (reviewed in Takayama and Isogai 2005). The stylar recognition component in this system is specified by the allele encoding the S-locus receptor kinase (*SRK*), while the pollen component is specified by the allele for the S-locus cystein-rich protein (*SCR*). The relationships among these taxa and the wealth of genomic information available in *Arabidopsis* make this system particularly advantageous for addressing questions about the mutation(s) that caused the transition to SC in *A. thaliana*, estimating when this transition took place, and dissecting events both at the S-locus and elsewhere in the genome that took place after the shift in compatibility system.

Many accessions of *A. thaliana* have a nonfunctional allele at the *SCR* locus, termed *SCR1*. Shimizu et al. (2004) found that sequence polymorphism within this allele was far lower than at many reference loci in *A. thaliana*. They estimated that the conversion to SC was very recent (>300,000 yr BP) and involved a selective sweep by the *SCR1* allele. It is interesting that polymorphism persists at the *SRK* locus, though it is much reduced relative to SI congeners. Three distinct *SRK* alleles were found, with levels of divergence among them similar to functional S-alleles in related SI species.

Bechsgaard et al. (2006) estimated how long the three alleles at *SRK* had been under neutral rather than diversifying selection. A switch to neutrality would occur on loss of SI. Again, conversion to SC was estimated as very recent (<420,000 yr BP). Neither molecular analysis (Shimizu et al. 2004; Bechsgaard et al. 2006) can reject 0 yr BP for the loss of SI. Analysis of *SRK* alleles in the SI species of *Arabidopsis* confirmed that they contain close orthologues of each of the three *SRK* alleles found in *A. thaliana* (Bechsgaard et al. 2006).

Nasrallah et al. (2004), however, suggested that additional polymorphism might also exist at the *SCR* locus in *A. thaliana* because the *SCR1* allele could not be detected by Southern blotting in 17 of 27 accessions tested. Polymorphism at *SCR* casts doubt on the hypothesis that a selective sweep by the nonfunctional *SCR1* allele caused the transition to SC, undermining the findings of Shimizu et al. (2004). Nasrallah et al. (2002, 2004) also transformed *A. thaliana*, inserting functional pollen-specificity (*SCR*) and style-specificity (*SRK*) alleles from *A. lyrata*. In one accession, full SI was restored, indicating that SC was due solely to mutations in the *SRK* and/or the *SCR* alleles. In another six accessions, such transformations failed to restore SI in mature flowers, though these transformed accessions showed varying levels of SI when pollinations were performed on developing flower buds (Nasrallah et al. 2004). The gene, which is S linked, that underlies variation in the timing and duration of the SI response following transformation has recently been cloned (Liu et al. 2007). Variants in this locus either represent additional mutations affecting SI that have accumulated in most populations subsequent to the loss of SI or may represent an ancestry in which most populations of *A. thaliana* exhibited transient (also called pseudo or partial) SI before conversion to full SC. Transient SI is the phenomenon in which flowers lose their SI response over time, allowing self-fertilization after opportunities for outcrossing have diminished (Levin 1996; Good-Avila and Stephenson 2002; Vallejo-Marin and Uyenoyama 2004).

Sherman-Broyles et al. (2007) analyzed the S-haplotype of the accession of *A. thaliana* that was restorable to SI following transformation. Surprisingly, this S-haplotype was derived from recombination between two different S-locus haplotypes. It is unclear whether the recombination event caused or followed the transition to SC; however, the date of the recombination event appears to have been recent (<200,000 yr BP; Sherman-Broyles et al. 2007).

The remaining issue of potential additional polymorphism at the *SCR* locus of *A. thaliana* has recently been addressed (Sherman-Broyles et al. 2007; Shimizu et al., forthcoming). It appears that several deletions are responsible for the failure of some European accessions of *A. thaliana* to provide a signal on Southern blots probed with the *SCR1* allele. Sequencing of the flanking regions shows that these deletions occurred in the *SCR1* allele and that 96% of 286 European accessions examined contain either the nonfunctional *SCR1* allele or its deletion

mutants (Shimizu et al., forthcoming). The remaining accessions fail to amplify when primers designed for the *SCR1* allele or its flanking regions are used, perhaps because of additional deletions, as in the recombinant haplotype that lacks *SCR* altogether (Sherman-Broyles et al. 2007; Shimizu et al., forthcoming). A different *A. thaliana SRK* allele, known from the Cape Verde Islands, is linked to a second *SCR* allele, indicating a possible independent origin of selfing. No *SCR* allele has yet been identified that is linked to the third *SRK* allele, which is more commonly found in Asian accessions. It is possible that variation in *SRK* reflects independent transitions to selfing in different glacial refugia (Mediterranean, Asia), with an additional origin of selfing in the Cape Verde Islands (Sherman-Broyles et al. 2007; Shimizu et al., forthcoming).

Taken together, these studies agree on several points. First, while some doubt remains about the causal mutations, conversion to SC in the lineage leading to *A. thaliana* appears to have occurred quite recently, within the past 0.5 million years, and perhaps as recently as the last glacial cycle (Shimizu et al. 2004). Therefore, the lineage leading to *A. thaliana* was probably SI for >90% of the time since the most recent ancestor shared with extant SI *Arabidopsis* species. Second, while polymorphism at the S-locus has not yet completely disappeared, it is certainly far reduced relative to related SI species, with only three alleles so far found at the style locus. Third, all the floral evolution considered part of the selfing syndrome displayed by *A. thaliana* (much-reduced flower size, pollen and stigma presentation positions and schedules that increase autogamy) would almost certainly have been detrimental before the loss of SI, implying rapid evolution of floral characters following transition to SC. Fourth, in addition to the nonfunctional *SCR* and *SRK* alleles, most populations have other mutations affecting SI (Nasrallah et al. 2004).

The primary effect of the loss of SI on the mating system of *A. thaliana* is difficult to ascertain because of extensive subsequent floral evolution resulting in the selfing syndrome. Even though many cases are known where homomorphic SI and SC plants coexist in populations (e.g., Tsukamoto et al. 1999; Mable et al. 2005; Stone et al. 2006), no studies compare the outcrossing rates of SI and SC forms in the same population. Studies of this sort would be useful because they would allow estimation of the direct effect of loss-of-function mutations on the mating system without the confounding effects of subsequent changes in floral form.

In contrast to its obligately outcrossing relatives, *A. thaliana* currently outcrosses ca. 1% of the time (Abbott and Gomes 1989). Individuals of *A. thaliana* are highly homozygous, and within-population nucleotide variation is lower in *A. thaliana* than in its SI sister group (Wright et al. 2003, 2007; Ramos-Onsins et al. 2004). However, species-wide variation in *A. thaliana* remains quite high (Nordborg et al. 2005), reflecting its broad geographic range and large species-wide population size. Single populations contain a substantial fraction (33%) of the total genetic variation found in the species, and populations within a region contain an additional 35% of the total variation. Whether persistence of large amounts of genetic variation within and among populations of *A. thaliana* is due to the recent shift to selfing, large local and species-wide population sizes, the complex history of postglacial colonization and admixture of populations, or other factors remains a topic of intense research (see Wright et al. 2007).

The genus *Solanum*, sect. *Lycopersicon* (the wild and the cultivated tomatoes), provides another excellent opportunity for studies of recent transitions to SC. The SI species in this group use the well-characterized S-RNase/F-box GSI mechanism (McClure 2006) that operates elsewhere in the Solanaceae. Repeated recent transitions to SC have occurred (fig. 2), with SI/SC polymorphism found among populations of all well-studied SI species. Among SC tomato species, a number of different defects in the SI mechanism are known (Kondo et al. 2002a, 2002b). All SC species examined have much-reduced levels of S-RNase activity in their styles, but some (*S. pimpinellifolium*, *S. cheesmaniae*, and *S. lycopersicum*) appear to lack the S-RNase gene altogether, while others (*S. neorickii*, *S. habrochaites*, and *S. chmielewskii*) have S-RNase alleles, though different ones in different species. All SC species display reduced transcription of the HT-B gene (Kondo et al. 2002a), which is unlinked to the S-locus but encodes a protein that is essential for SI in Solanaceae (McClure et al. 1999). Because the phylogenetic relationships among SI and SC species are difficult to resolve with certainty (Spooner et al. 2005) and the species in question may hybridize, it may be too early to propose a scenario to explain the order of events leading to SC in all species and populations. Nevertheless, the multiple defects seen in many taxa and the limited implied age of SC in any of these lineages indicate that additional mutations accumulate once SI is lost.

Several transitions from obligate or predominant outcrossing to selfing are thought to have occurred in the tomato group (fig. 2), but few quantitative studies of outcrossing rates have been performed. Rick et al. (1978) experimentally confirmed low rates of outcrossing in *S. pimpinellifolium* (mean $t = 0.135$), which is almost entirely selfing in the southern part of its range, with some large-flowered individuals from the northern part of the range exhibiting outcrossing rates as high as $t = 0.4$. Based on its diminutive flower size, very low stigma exertion, and autogamy in glasshouse-grown individuals, it is commonly thought that *S. neorickii* is also primarily selfing. The cultivated tomato (*S. lycopersicum*) and its close relatives (*S. cheesmaniae* and *S. galapagensis*) each share similar selfing attributes.

Convergence on the selfing syndrome following loss of SI appears to be rapid. This small group shows correlated variation in mating systems and flower sizes not only among closely related species but also within species, where interpopulation variation in SI status is found. For example, two populations in the principally SI *S. habrochaites*, which occur at the northern and southern geographical limits of the species, independently acquired SC (Rick et al. 1979; Rick and Chetelat 1991). These SC populations also show marked reductions in flower size. Similarly, a SC accession of *S. arcanum* (LA2157) has smaller flowers than other accessions from this species (Rick 1982). It seems that the breakdown of SI can be rapidly followed by the evolutionary transition to selfing.

Recent studies of the population genetic consequences of transitions to SC in the tomato group (Baudry et al. 2001; Roselius et al. 2005; Städler et al. 2005) find far more striking reductions in genetic variation between SI and SC taxa than has been found in *Arabidopsis*. For instance, silent nucleotide diversity in the SC and partially selfing *S. pimpinellifolium* and *S. chmielewskii* was less than half that of the three SI species examined (*S.*

peruvianum, *S. habrochaites*, and *S. chilense*; Roselius et al. 2005). Because complete selfing theoretically reduces N_e by a factor of only one-half relative to obligate outcrossing, demographic and ecological shifts in addition to the transition in mating system must account for some of these reductions in genetic variability. Roselius et al. (2005) examined only single-population samples of each species, so species-wide effects remain unknown. Opportunities abound to document both population- and species-wide shifts in patterns of genetic variation associated with transitions from SI to SC in *Solanum* sect. *Lycopersicon*.

Several studies find interpopulation or interindividual variation in the presence or strength of SI (Tsukamoto et al. 1999; Good-Avila and Stephenson 2002; Mable et al. 2005; Stone et al. 2006). In some cases, SI may be best characterized as a quantitative trait whose strength is affected by various genetic factors, both linked and unlinked to the *S*-locus. Under some conditions, partial SI may be evolutionarily stable (Vallejo-Marin and Uyenoyama 2004). However, the effect of variation in the presence or strength of SI on the outcrossing rate of populations has rarely been measured. In perhaps the most complete study, Mable et al. (2005) found variation within and among *A. lyrata* populations from the southern Great Lakes region of North America in the ability of plants to set fruit and seed on selfing. They also estimated the outcrossing rates of populations that displayed different frequencies of self-fertile individuals, a unique practice among studies of this type. Populations with higher frequencies of SC in glasshouse studies showed markedly lower outcrossing rates and reduced genetic diversity than more SI populations. Three populations with low frequencies of SC individuals had outcrossing rates not statistically different from 1. Two populations, in which the frequency of SC was much higher, had outcrossing rates well below 0.5. Because no differences in flower size were noted, this would appear to reflect differences in the mating system resulting from differences in the presence or strength of SI, though differences among populations in pollination services may also play a role. Because even the more selfing populations contained substantial fractions of individuals that were strongly SI, this is a minimum estimate of the difference in individual outcrossing rate caused by a switch to SC in this species.

The existence of populations containing individuals that vary in the presence or strength of SI brings the inevitable question of whether such populations represent stable polymorphisms (and stable mixed mating) or whether they represent transitory situations in which genes causing SC will either disappear following their temporary invasion or will spread to fixation. Porcher and Lande (2005b) showed theoretically that mutations causing SC readily invade GSI populations under many parameter combinations. However, the conditions for stable polymorphisms are much more restrictive than conditions for fixation. Given the number of parameters involved in their model, empirical demonstration of the stability of mixed populations appears unlikely, though experiments on the fitness of SC individuals when rare and frequent might be a reasonable avenue of future research.

Several authors studying mixed populations of SI and SC individuals (e.g., Brennan et al. 2006; Stone et al. 2006) have pointed out that the balance between colonization ability of SC forms and the increased fitness of outcrossed offspring following population establishment could lead to the frequent observation of mixtures of SI and SC individuals within populations. Metapopulation models (Pannell and Barrett 1998; Schoen and Busch 2007) indicate that selection for reproductive assurance intensifies whenever local population persistence, the number of migrants to unoccupied sites, or the proportion of occupied sites is low. Therefore, SC forms would be more likely to occur and persist in otherwise SI species wherever local population turnover is high.

Conclusions

While mixtures of SI and SC individuals may be observable in many species, the phylogenetic record of well-characterized SI mechanisms indicates the frequent, complete, and irreversible loss of SI. The evidence for irreversibility is broadly shared ancestral polymorphism at the *S*-locus among SI species and genera in various families (Castric and Vekemans 2004; Igic et al. 2004, 2006). This implies a continuous ancestry of SI for all SI species sharing ancestral *S*-locus polymorphism. We show that SC species, even those recently derived from an SI ancestor, often harbor severely reduced variation at the *S*-locus. Most SC populations and some species are fixed on a single *S*-allele despite these recent transitions to SC. They also tend to harbor multiple loss-of-function mutations, making reversion to SI difficult. Because of the loss of *S*-locus diversity, recovery of the same SI mechanism would leave a genealogical imprint on the *S*-locus for tens of millions of years. To date, only one example of a severe historical bottleneck at the *S*-locus is known, the bottleneck shared by the Solanaceae genera *Witheringia* and *Physalis* (Richman et al. 1996; Richman and Kohn 1999, 2000; Lu 2001; Stone and Pierce 2005). In that case, three or four allelic lineages predate the bottleneck, providing no evidence that SI was ever completely lost.

A large fraction of angiosperms are SI, even if the current estimate of the frequency of SI species suffers from bias. Coupled with the evidence for frequent transitions to SC, the high frequency of SI among angiosperm species suggests that SI provides a macroevolutionary advantage. It is probably not a coincidence that in both *Arabidopsis* and the tomatoes, evidence of multiple recent transitions to SC from SI ancestors is found. If frequent transitions have been the rule since the origin of each SI mechanism, nearly all species would be expected to be SC in the absence of differences in net diversification rates between SI and SC lineages.

Clearly, not all SC lineages are doomed, particularly when they do not shift to predominant self-fertilization. This is best supported by the evidence for the evolution of nonhomologous SI systems and the existence of relatively large and old monophyletic groups that seem to lack SI altogether (e.g., the Cucurbitaceae). Where sufficient rates of outcrossing can be maintained by alternative mechanisms, SC lineages may proliferate. In addition, shifts from increased to decreased rates of self-fertilization are known, if not particularly frequent, in the literature (Barrett and Shore 1987; Olmstead 1990; Takebayashi and Morrell 2001). However, we know of no large and old angiosperm families where self-fertilization is the predominant mode of reproduction.

Our simple model posits unidirectional mating system shifts from obligate outcrossing (SI) to predominant outcrossing and then to predominant selfing. It is capable of producing a variety

of distributions of breeding-system states among species even if the direction of mating system change, once SI is lost, usually leads to selfing. For too long the U-shaped distribution (a dearth of mixed-mating species with many outcrossers and selfers) has been the main issue discussed in studies of the distribution of outcrossing rates. Given variation in diversification rates among lineages with different mating systems, a variety of distributions is possible, and symmetric bimodality is, in fact, rather unlikely.

Acknowledgments

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Note Added in Proof

A recent article by Tang et al. argues that the transition from SI to SC in *A. thaliana* is considerably older than previous reports indicate. (Tang C, C Toomajian, S Sherman-Broyles, V Plagnol, Y-L Guo, TT Hu, RM Clark, et al 2007 The evolution of selfing in *Arabidopsis thaliana*. Science 317:1070–1072.)