reduction in chemical potential by forming the crystalline phase. In the second scenario, we assume that oxygen has first adsorbed to the LS interface, driven by the reduction in interface energy (*24*, *28*), having diffused along the interface from the triple junction. Once oxygen adsorbs, a critical-size nucleus (in the form of a step) forms at the triple junction and sweeps across the LS (0001) interface, forming a new (0006) layer of α-Al$_2$O$_3$ in its wake. Oxygen then again adsorbs to the new LS interface, supplied from the dissolving outer rim of the nanowire. The nucleation rate of steps is relatively low under the present experimental conditions, leading to single steps that sweep across the interface (no defects resulting from the intersection of steps or multiple nucleation events were detected). Both of these reactions would be very fast; the formation of a new (0006) layer after oxygen diffusion has started requires only ~0.16 s. Our experimental observations cannot distinguish between these two scenarios.

Our in situ HRTEM observations have revealed an oscillatory growth process that is not obvious from ex situ observations of grown nanowires. These observations provide an explanation for the (0001) growth mechanism and why it is not continuous. What distinguishes this reaction from most VLS processes is that the catalyst droplet is not directly involved in supplying all the necessary components for growth. Instead, oxygen for the new (0006) layer is supplied by transient sacrificial dissolution of the top rim of the nanowires via facet formation. The components of interface tensions at the triple junction also promote formation of the facets and probably drive the nucleation of steps of solid (0006), which sweep across the LS (0001) interface, resulting in growth of a new (0006) layer. Formation of the (0006) layer is relatively fast because the Al atoms on the liquid side of the LS surface

are already in positions similar to those in the underlying bulk sapphire. The oxygen collected on the LV surface is used to eradicate the facets at the outer, top rim of the nanowires once a complete (0006) layer is formed. It is this process that is the rate-limiting step.

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Species Selection Maintains Self-Incompatibility

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Identifying traits that affect rates of speciation and extinction and, hence, explain differences in species diversity among clades is a major goal of evolutionary biology. Detecting such traits is especially difficult when they undergo frequent transitions between states. Self-incompatibility, the ability of hermaphrodites to enforce outcrossing, is frequently lost in flowering plants, enabling self-fertilization. We show, however, that in the nightshade plant family (Solanaceae), species with functional self-incompatibility diversify at a significantly higher rate than those without it. The apparent short-term advantages of potentially self-fertilizing individuals are therefore offset by strong species selection, which favors obligate outcrossing.

The astonishing diversity of flowering plants has led to numerous hypotheses linking the propensity for diversification to morphological, life history, and physiological char-

acteristics (*1*–*4*). Although it is clear that no single character can entirely explain the variation in plant diversity (*2*, *4*), many traits potentially associated with an increase in species richness

may have been overlooked because of methodological shortcomings (*5*, *6*).

The overwhelming majority of flowering plants function as both males and females, but despite the potential for self-fertilization in hermaphrodites, most of these plants predominantly or exclusively mate with other individuals (outcross) (*7*, *8*). Outcrossing is often enforced by self-incompatibility (SI): the ability of individual plants to recognize and reject their own pollen. Each SI system characterized to date relies on

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complex genetic mechanisms (9). Their value is primarily attributed to the avoidance of inbreeding depression (10), but the short-term disadvantages of SI, which limits an individual plant's ability to reproduce if outcross pollen is not available, is expected to result in frequent transitions to self-compatibility (SC) (8). Mate limitation and the automatic advantage of selfing (10, 11) each favor the spread of mutants that break down SI. Indeed, angiosperm families in which SI is found also contain significant proportions of SC species (12). The two states are commonly interspersed within clades, implying frequent evolutionary transitions. Independent data, both genetic and phylogenetic (12, 13), suggest that the transition from SI to SC is one of the most common evolutionary shifts in flowering plants (14). Studies of the genetic basis of SI indicate that novel SI systems originate very rarely (9, 15). The combined evidence implies that SI is frequently lost by species and that an identical form of SI is only rarely, if ever, regained (12).

In Solanaceae species with SI, strong negative frequency-dependent selection maintains dozens of alleles at the locus controlling self-recognition (S-locus), where it has preserved ancestral polymorphism over 40 million years (13, 16, 17). After pollination, if the haploid pollen allele matches either allele of the diploid female reproductive organ, fertilization is prevented. Individuals with common alleles therefore have fewer potential mates, and those with rare alleles more, so that alleles ordinarily lost by drift are instead maintained over long time scales (16). The outcome is a pattern of ancestral polymorphism shared not only across species boundaries but among distantly related genera. Randomly sampled alleles from different SI species of Solanaceae are often more closely related than those within species (16, 17). This provides strong evidence that a polymorphic and therefore functional S-locus was present in the common ancestor and passed on to descendent SI species in the family (17).

Although the SI mechanism has been frequently lost and not regained within Solanaceae (13), it occurs at an intermediate frequency. The proportion of SI species is declining with each transition to SC, so SI may be destined for extinction. But the SI mechanism of Solanaceae is ~90 million years old, nearly as old as eudicots (15), and SI may instead persist if the diversification rate of SI lineages exceeds that of SC lineages by at least the SI-to-SC transition rate (17). In this scenario, the loss of SI may be countered by a greater difference between speciation and extinction rates associated with the presence of SI relative to SC. A strong association between SI and increased diversification would provide unusually compelling evidence for a causal relationship, because frequent character changes to SC uncouple the evolution of the focal character from others and reduce the chance that an inferred connection is spurious (18).

We examined the effect of SI on diversification in Solanaceae. The nightshades contain many agriculturally important species (such as tomato, potato, chile, and tobacco), so phylogenetic and breeding system data are extensive. Among the estimated 2700 species in the family, approximately 57% are SC, 41% are SI, and less than 2% have separate male and female plants (19).

We constructed a phylogeny through an initial alignment matrix of 998 species and eight genes, and we assigned character states (SI or SC) for 616 species (19). Subsequent analyses concentrated on a monophyletic clade termed "x = 12" for its uniform base chromosome number, containing approximately 2300 species, including the subfamilies Solanoideae and Nicotianoideae (20) [we also conducted analyses on the whole family (19)]. Both character states and phylogenetic data are better sampled in this clade than in the rest of the family, yielding 356 species present in the tree and with known mating systems

(Fig. 1). This sample did not statistically differ in SI frequency from the total available data set of 616 species ($P > 0.3$), and our analyses accounted for sampling incompleteness (21).

Irreversible loss of SI in Solanaceae will cause the frequency of SI to decline to zero if $r_I \leq r_C + q_{IC}$, where r_I and r_C are net diversification rates (the difference between speciation and extinction rates) for SI and SC lineages, respectively, and q_{IC} is the per-lineage rate of transition from SI to SC. Alternatively, the loss of SI may be offset by more rapid diversification of SI lineages when $r_I > r_C + q_{IC}$. In this latter case, the equilibrium proportion of SI species, p_{SI}, is positive and equal to $1 - q_{IC}/(r_I - r_C)$ (17).

We analyzed character evolution and diversification on the maximum likelihood tree (Fig. 1) and also on 100 bootstrap trees to assess robustness to phylogenetic uncertainty (19). Rates of speciation (λ_I and λ_C; the subscripts denote SI
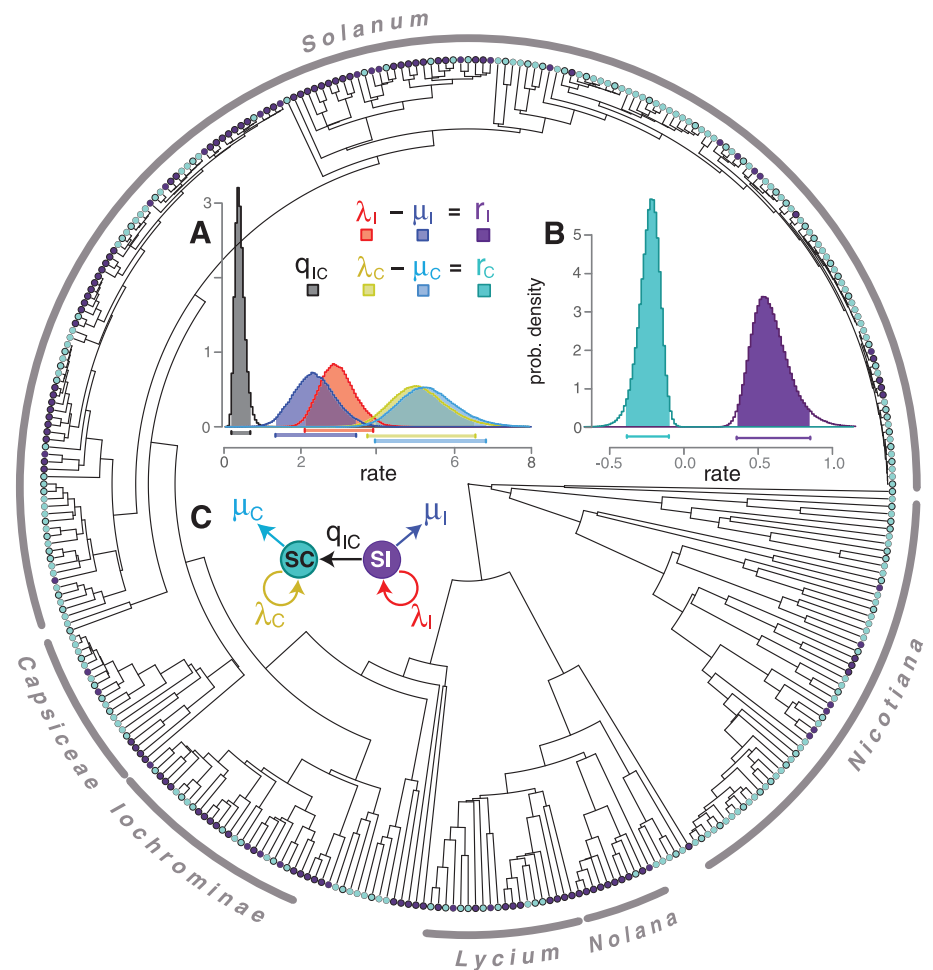

Fig. 1. Maximum likelihood tree of phylogenetic relationships among 356 species of Solanaceae. Higher ranks are indicated around the perimeter of the tree. Purple and turquoise tip colors denote SI and SC extant species, respectively. The root age is 36 million years. Inset panels display posterior probability distributions and 95% credibility intervals of reconstructed rates of character evolution (the time unit is millions of years). (**A**) BiSSE estimates of transition, speciation, and extinction parameters ($q_{IC} << \mu_I < \lambda_I << \lambda_C < \mu_C$). (**B**) Net diversification rate—the difference between speciation and extinction rates—associated with each state. (**C**) Schematic summary of estimated rate parameters. For methods, species names, character states, and further results, see (19).

and SC states, respectively), extinction (μ_I and μ_C), and loss of SI (q_{IC}) were estimated with Markov chain Monte Carlo analyses under the binary-state speciation and extinction (BiSSE) model (*19, 22*), which explicitly incorporates differing speciation and extinction rates associated with the two states of a binary character. Because SI has apparently not been regained in this clade (*13*), we fixed the rate of transitions from SC to SI, q_{CI}, to be zero in our main analyses [although we also considered bidirectional transitions (*19*)].

We find a higher rate of speciation for SC than SI lineages, but this apparent advantage is erased by an SC extinction rate that exceeds that of SI lineages and even the SC speciation rate [$\mu_I < \lambda_I < \lambda_C < \mu_C$, with posterior probability 0.99 (Fig. 1A)]. SI lineages consequently show a substantially higher rate of net diversification than do SC lineages [$r_I > r_C$, with posterior probability 1.0 (Fig. 1B)]. Furthermore, this diversification difference is large enough to counter the irreversible loss of SI [$r_I > r_C + q_{IC}$, with posterior probability 1.0 (Fig. 2A)]. The equilibrium frequency of SI is therefore positive [$p_{SI} > 0.25$, with posterior probability 0.99 (Fig. 2B)], demonstrating that the macroevolutionary advantage of SI is enough for it to persist indefinitely, despite its loss through transitions to SC. These results are robust to phylogenetic uncertainty and against the assumption that SI is not regained (*19*).

The higher rate of diversification associated with SI means that selection is acting among species, which can produce trends that are not predictable from studies of selection among individuals within species. Natural selection can act on many levels of evolutionary hierarchy (including genes, individuals, populations, and species), with the potential for cascading effects of selection from any level to those above and below (*18, 23–26*). Species, as units, may vary in heritable traits associated with different rates of multiplication. A higher form of selection may thus be manifested as a trait-dependent net diversification rate. There is wide acceptance of the existence of higher-order selection (*18, 25, 26*) but relatively sparse data on its importance (*25, 26*).

Our results point to a strong role for species selection in determining the evolutionary dynamics and distribution of mating systems among species. Both theoretical and empirical studies on the shape of outcrossing rate distributions have overlooked the possibility that species selection may produce an arbitrary proportion of selfing, mixed-mating, and outcrossing species (*8, 27*).

It remains unknown why strict avoidance of self-fertilization yields such strong long-term evolutionary advantages. Outcrossing plants, which generally maintain high effective population sizes and recombination rates, may have increased polymorphism, lower linkage disequilibrium, more efficient response to purifying selection, more extensive geographic distribution of genetic diversity, and lower rates of extinction (*28–31*). Various mechanisms other than SI, such as the physical and temporal separation of sexes within hermaphrodite flowers, or single-sex individuals, can effect outcrossing in the absence of SI (*3*). Moreover, although SI may be a relatively efficient mechanism for obligate outcrossing (*32*), delayed selfing ability and plastic sex expression afforded by other breeding systems may additionally provide reproductive assurance (*7*).

At short time scales, the expression of inbreeding depression can oppose the spread of mutations that break down SI, but many ecological factors favor their rapid and frequent fixation (*7*). This is problematic because natural selection cannot maintain features slated for future use (*18*). We find that SC is associated with a macroevolutionary disadvantage from high extinction rates, despite its high speciation rates relative to SI. Both theoretical and empirical evidence link inbreeding with an increased probability of extinction (*27, 29, 31*). Self-fertilization and increased inbreeding are also closely tied to higher among-population differentiation (*30*), which may in turn lead to escalating speciation rates.

We conclude that species selection opposes the short-term advantages provided by the ability of flowering plants to self-fertilize, and that volatile dynamics underlie the scattered phylogenetic distribution of SC plant species. A strong relationship between traits, including SI, and speciation and extinction may result in the uneven patterns of diversification rates observed among clades of flowering plants (*2, 4*), especially when coupled with time heterogeneity and historical contingency. These results provide evidence that the evolutionary origin and maintenance of SI may play an important role in shaping the immense extant diversity of flowering plants (*18, 33*).

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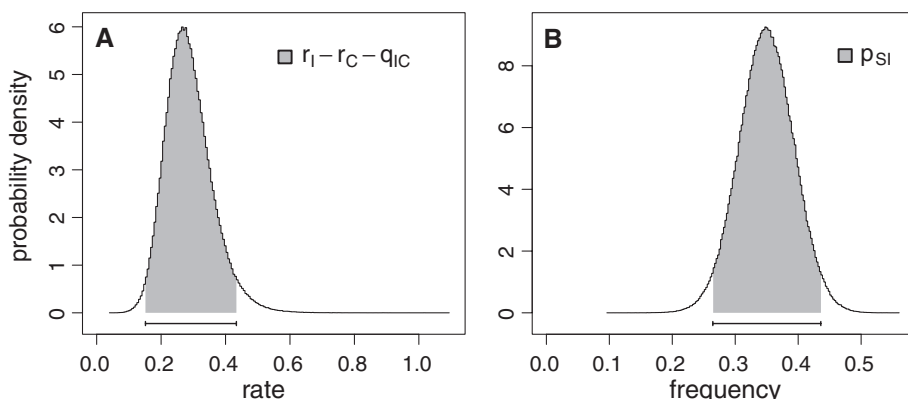
Fig. 2. Further analyses of the posterior rate distributions in Fig. 1. (**A**) The diversification rate of SI is sufficiently higher than that of SC for it to persist deterministically (shown by positive values here) despite frequent and irreversible loss. (**B**) The equilibrium frequency of SI is consequently always positive.