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The population genetics of sporophytic self-incompatibility in *Senecio squalidus* L. (Asteraceae): avoidance of mating constraints imposed by low *S*-allele number

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Senecio squalidus L. (Asteraceae) has been the subject of several ecological and population genetic studies due to its well-documented history of introduction, establishment and spread throughout Britain in the past 300 years. Our recent studies have focused on identifying and quantifying factors associated with the sporophytic self-incompatibility (SSI) system of *S. squalidus* that may have contributed to its success as a colonist. These findings are of general biological interest because they provide important insights into the short-term evolutionary dynamics of a plant mating system. The number of *S*-alleles in populations and their dominance interactions were investigated in eight wild British populations using cross-diallel studies. The numbers of *S*-alleles in British *S. squalidus* populations are typically low (average of 5.3 *S*-alleles) and the entire British population is estimated to possess no more than 7–11 *S*-alleles. Such low numbers of *S*-alleles are most probably a consequence of population bottlenecks associated with introduction and colonization. Potential evolutionary impacts on SSI caused by a paucity of *S*-alleles, such as restricted mate availability, are discussed, and we suggest that increased dominance interactions between *S*-alleles may be an important short-term means of increasing mate availability when *S*-allele numbers are low.

Keywords: sporophytic self-incompatibility; *Senecio squalidus*; *S*-allele; population bottleneck; mate availability

1. INTRODUCTION

Self-incompatibility, where self-pollen is recognized and prevented from germinating or growing through pistil tissues, is a widespread mechanism promoting outcrossing and gene flow in angiosperms (Hiscock & Kües 1999). In general, SI systems function through the expression of pollen (male) and pistil (female) genes residing at a single *S*-locus and an incompatibility reaction occurs when plants express the same, allelic, pollen and pistil *S* factors (Hiscock & Kües 1999). *S* loci are usually highly polymorphic because negative frequency-dependent selection will favour any new or rare *S*-alleles due to their compatibility with more mates (Wright 1939). SI systems are classified according to whether the male *S* factor is autonomously expressed in pollen (GSI) or in the paternal diploid anther (SSI). These differences in the expression of the male *S* factor 'in pollen' between GSI and SSI systems have important reproductive consequences. In SSI systems, complex *S*-allele dominance interactions are possible because of diploid control of pollen incompatibility, so that *S*-alleles can show (i) complete dominance; (ii) partial dominance; or (iii) tissue-specific dominance interactions in either pollen or stigma (Wallace 1979). *S*-allele dominance masks the phenotypic effects of recessive *S*-alleles leading to (i) higher frequencies of recessive *S*-alleles relative to dominant *S*-alleles; (ii) more potentially compatible mates in populations; and (iii) the natural occurrence of *S* homozygous individuals (Byers & Meagher 1992; Vekemans *et al.* 1998).

Senecio squalidus L. (Asteraceae) is a weedy, short-lived perennial native to Sicily that has a well-documented history of introduction, establishment and colonization in Britain (Harris 2002). Unusually for a colonizing species, *S. squalidus* is strongly self-incompatible. Self-incompatible species have generally been regarded as less effective colonizers than self-compatible species because of reproductive assurance constraints (Baker 1967; Abbott & Forbes 1993). In common with other members of the Asteraceae, SI in *S. squalidus* is sporophytic (Hiscock 2000*a*,*b*).

2. ESTIMATES OF *S*-ALLELE NUMBER AND DOMINANCE RELATIONSHIPS IN UK *S. SQUALIDUS* POPULATIONS

Preliminary investigations suggested that UK populations of *S. squalidus* contain very few *S*-alleles compared to other wild populations of species with SSI (Hiscock 2000*a*,*b*). A more extensive survey of *S*-alleles in an Oxford population confirmed this prediction by ident-

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Table 1. Percentage of observed *S*-allele dominance interactions from wild sampled populations of *S. squalidus* and other species with SSI.

species (family)	no. of populations studied	no. of *S*-alleles studied[a]	no. of different *S*-alleles observed[b] (mean/population)	completely dominant alleles[c]	stigma-dominant alleles[c]	pollen-dominant alleles[c]	co-dominant alleles[c]
Senecio squalidus (Asteraceae)	8	138	7–11 (5.3)	89.0	3.3	3.3	4.4
Brassica campestris[d] (Brassicaceae)	1	24	15	0.0	3.3	13.3	83.3
Raphanus sativus[e] (Brassicaceae)	2	65	18 (12.5)	67.3	4.1	18.4	10.2
Sinapis arvensis[f] (Brassicaceae)	1	70	35	2.4	4.8	50.0	42.8
Ipomoea trifida[g] (Convolvulaceae)	6	448	49 (13.7)	89.9	0.9	5.2	4.0

[a] Number of *S*-alleles studied calculated as the sum of one allele per individual where only one dominant allele is observed, and two alleles per individual where co-dominance is observed or both alleles are identified in progeny arrays.
[b] Number of different *S*-alleles observed calculated as the total number of different *S*-alleles identified through compatible cross-interactions between individuals in cross-diallels.
[c] Percentages completely dominant, co-dominant and tissues-specific co-dominant *S*-alleles calculated relative to the total number of *S*-alleles studied.
[d] Data from Nou *et al.* (1991).
[e] Data reanalysed from Karron *et al.* (1990) according to the methods described in Brennan *et al.* (2002).
[f] Data from Stevens & Kay (1989).
[g] Data from Kowyama *et al.* (1994).

ifying just six *S*-alleles in 26 plants, and a total of six to seven *S*-alleles was estimated for the entire Oxford population using population *S*-allele estimators modified for SSI (Brennan *et al.* 2002). Current estimates of *S*-allele number for a further seven UK populations from Cornwall (St Blazey) to Scotland (Kirriemuir) revealed an average of 5.3 *S*-alleles per population with no single population having more than six *S*-alleles (see table 1 in Brennan 2003). Preliminary compatibility data from cross-checking *S*-allele identities between the different sampled populations suggest that a total of no more than seven to 11 *S*-alleles are present in the entire UK population (Brennan 2003). The total is probably closer to seven than 11, because there is uncertainty associated with the inter-population classification of one *S*-allele, *S7*. *S*-allele tester plants representing *S7* were unavailable for the Oxford reference population so insufficient inter-population crosses were carried out to confirm whether this *S*-allele is the same one in all populations that may contain it. Since all other *S*-alleles are largely shared between populations, it is reasonable to assume that the additional *S*-allele identified in different populations may represent the same *S*-allele.

The number of *S*-alleles identified in British *S. squalidus* populations is far fewer than the number of *S*-alleles identified in other studies of wild species with SSI (figure 1). Low numbers of *S*-alleles in *S. squalidus* relative to other species with SSI is most probably a direct consequence of *S. squalidus*' history of introduction, establishment and colonization in Britain over the past 300 years (Hiscock 2000*a*; Harris 2002; Brennan *et al.* 2002). No obvious geographical pattern to the distribution of *S*-alleles was observed for the eight British populations studied, therefore inter-population migration and strong negative frequency-dependent selection favouring new migrant *S*-alleles have probably already been sufficient to obscure any patterns of population *S*-allele numbers associated with the colonization history of *S. squalidus*.

3. MATING CONSEQUENCES FOR SI SYSTEMS WITH FEW *S*-ALLELES

Small numbers of *S*-alleles within populations reduce mate availability and pose a threat to reproductive assurance (Byers & Meagher 1992; Vekemans *et al.* 1998). In Asteraceae species that have experienced dramatic population bottlenecks similar to those encountered by *S. squalidus*, reduced numbers of *S*-alleles arising as a consequence of the bottleneck, have been found to limit successful sexual reproduction (DeMauro 1993; Reinartz & Les 1994). By contrast, *S. squalidus* appears to have overcome any potential problems of reduced mate availability arising from restricted numbers of *S*-alleles because seed set in the wild always appears consistently high (A. Brennan, personal observation).

One possible means, for a self-incompatible species, of ensuring reproductive success following a bottleneck is to evolve SC or pseudo-self-compatibility (Levin 1996). This is happening in the rare North American endemic, *Aster furcatus* (Reinartz & Les 1994), where SSI is breaking down in favour of SC, but there is no evidence for SSI breakdown in *S. squalidus* (Abbott & Forbes 1993; Hiscock 2000*a*; Brennan *et al.* 2002). Pseudo-self-compatibility has been detected at low frequency in wild *S. squalidus* populations but, despite the potential ease with which a self-compatible or pseudo-self-compatible phenotype could spread through a population, the selective balance between reproductive assurance and prevention of inbreeding depression continues to favour SI over SC in *S. squalidus* (Hiscock 2000*a*; Brennan *et al.* 2002).

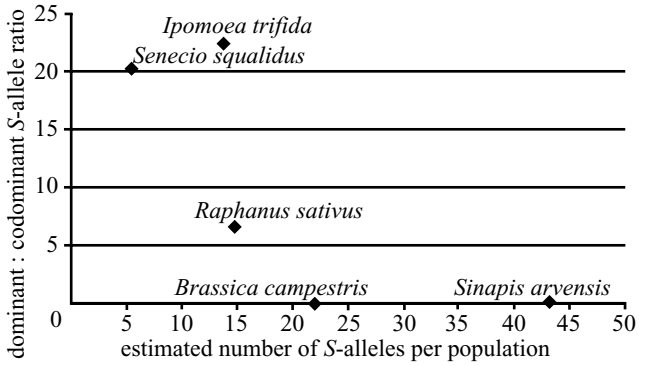

Figure 1. Relationship between estimated population *S*-allele diversity and *S*-allele dominance interactions for four species with SSI. Data for species other than *S. squalidus* from the same sources as referenced in table 1; *x*-values calculated according to Paxman (1963) and based on the *S*-allele data presented in table 1; *y*-values calculated as a proportion of fully dominant alleles/proportion of fully co-dominant alleles as presented in table 1.

The evolution of new *S*-alleles could also alleviate reduced mate availability, but such events are predicted to be extremely rare, because mutation must cause a change in both the pollen *S* factor and stigma *S* factor to preserve effective SI (Charlesworth 2000). The evolution of new *S*-alleles in *S. squalidus* over a period of only approximately 300 years therefore seems very unlikely, so alternative explanations must be sought to account for its reproductive success. Three possible explanations of how *S. squalidus* avoids potential mating constraints imposed by its low number of *S*-alleles are discussed below.

(a) *Reproductive features independent of the SI system*

Spatial autocorrelation of allozyme diversity and ecological observations of wild Oxford *S. squalidus* populations revealed little evidence for spatial substructure of *S*-alleles and allozymes for individuals separated by up to 0.48 km in an Oxford population of *S. squalidus* (Brennan *et al.* 2003). This shows that ample mating opportunities exist for *S. squalidus* individuals at scales of this magnitude, possibly as a consequence of life-history factors favouring effective pollen and fruit dispersal and multiple mating opportunities. Observations made over the course of a full flowering season for six Oxford populations of *S. squalidus* highlighted a long flowering season, utilization of generalist insect pollinators, wind-dispersed seeds and high population density as life-history features that probably contribute to its reproductive success in the UK (A. C. Brennan, S. A. Harris and S. J. Hiscock, unpublished data).

(b) *Modifier loci*

Flexibility in expression of SI can be achieved through the action of modifier loci, not linked to the *S*-locus, and it has been suggested that *S* modifier loci can temporarily preserve a functional SI system in species that have lost *S*-alleles (Levin 1996). There is much evidence for the activity of modifier loci affecting the compatibility relationships between individuals of *S. squalidus*, where their effect is to generally increase the number of compatible crosses between individuals sharing an *S*-allele, without affecting SI *sensu strictu* (Hiscock 2000*b*; Brennan *et al.* 2002). Two types of modifier appear to affect compatibility relationships in *S. squalidus*. One type produces its effect by changing the dominance relationships between *S*-alleles, while the second seems to act completely independently of the *S*-locus causing compatibilities that cannot be explained by any variant of the sporophytic model (D. A. Tabah and S. J. Hiscock, unpublished data). This latter class of modifier may be equivalent to the gametophytically expressed *G* gene identified by Lewis and coworkers in *Brassica campestris* and *Raphanus sativus* (reviewed in Lewis 1994). This diallelic gene was proposed to regulate a cryptic, possibly ancient, system of GSI operating alongside the SSI system of these species, the effects of which were only revealed in certain *S*-allelic backgrounds. Work is currently under way to test for the presence of this *G* gene system in *S. squalidus*.

(c) *Increased dominance interactions between S-alleles*

S-allele dominance interactions can increase mate availability when *S*-allele diversity is diminished, by permitting compatible crosses between individuals sharing recessively expressed *S*-alleles (Byers & Meagher 1992; Vekemans *et al.* 1998; Brennan *et al.* 2002). We have therefore speculated (Brennan *et al.* 2002, 2003) that there has been selection for newly evolved *S*-allele dominance interactions in British *S. squalidus* in response to low *S*-allele number because it increases mate availability and reproductive assurance. High levels of dominance certainly define all British *S. squalidus* populations sampled to date (table 1) and most *S*-allele dominance is complete in both pollen and stigma, allowing individuals to be identified by the presence of a single completely dominant *S*-allele (Brennan 2003). Nevertheless, tissue-specific *S*-allele dominance is also a fairly regular feature of the SSI system of *S. squalidus* where equal frequencies of pollen-dominant and stigma-dominant *S*-alleles were observed, contrary to the bias for pollen-specific dominance characteristic of species in the Brassicaceae (table 1).

Our preliminary investigations of *S*-allele dominance interactions in *S. squalidus* suggest an apparently contradictory pattern of high frequencies of fully dominant *S*-alleles within populations (table 1), but co-dominance and tissue-specific dominance interactions of *S*-alleles between populations (A. C. Brennan, S. A. Harris and S. J. Hiscock, unpublished data). Cross checking *S*-allele identity between populations suggests that all *S*-alleles so far identified are potentially co-dominant with each other (A. C. Brennan, S. A. Harris and S. J. Hiscock, unpublished data). This pattern of high levels of *S*-allele dominance within populations, and co-dominance of *S*-alleles between populations, shows that *S*-allele dominance interactions do vary between different British *S. squalidus* populations. This population-specific *S*-allele dominance may be the result of recent widespread selection for increased *S*-allele dominance among an originally mainly co-dominant set of *S*-alleles introduced with *S. squalidus*. Independent evolution of *S*-allele dominance within the different populations would explain why different British populations are characterized by different *S*-allele dominance interactions. The next logical step for these studies

of *S*-allele dominance in *S. squalidus* is to carry out comparable analyses on Sicilian populations that are known as the source of material introduced to the British Isles.

The factor controlling dominance interactions in *S. squalidus* could be a part of the non-recombining *S*-locus so that particular *S*-allele dominance interactions are always associated with particular *S*-allele pairs. Alternatively, unlinked *S* modifier loci may act to introduce or alter *S*-allele dominance interactions as is suggested by our preliminary studies of the inheritance of a pollen and stigma recessive *S*-allele in inbred *S. squalidus* lines (D. A. Tabah and S. J. Hiscock, unpublished data).

4. COMPARISON OF *S*-ALLELE DOMINANCE INTERACTIONS IN *S. SQUALIDUS* AND OTHER SPECIES WITH SSI

When frequencies of *S*-allele dominance in *S. squalidus* populations are compared with those from wild populations of other species with SSI an interesting pattern emerges (table 1). Frequencies for each kind of dominance interaction observed in *S. squalidus* are very similar to those observed in *Ipomoea trifida* (Convolvulaceae), with high frequencies of fully dominant and low frequencies of co-dominant and tissue-specific dominant *S*-alleles. By contrast, *Brassica campestris* and *Sinapis arvensis* (Brassicaceae) exhibit higher frequencies of co-dominant *S*-alleles, fewer fully dominant *S*-alleles, and characteristically higher frequencies of pollen-dominant *S*-alleles (table 1).

These differences in the frequencies of particular *S*-allele dominance interactions between wild species with SSI may reflect independently evolved SSI systems—one system of SSI being common to the Asteraceae and Convolvulaceae and a different system operating in the Brassicaceae. Alternatively, the observed frequencies may simply reflect the outcome of selection for altered *S*-allele dominance interactions to maximize within-population mate availability—more intense selection for increased mate availability through increased dominance being predicted in populations with fewer *S*-alleles (Byers & Meagher 1992; Vekemans *et al.* 1998). Indeed, our preliminary observations of *S*-allele dominance in *S. squalidus* and estimated numbers of *S*-alleles in populations of five species with SSI (figure 1) appear to support this relationship.

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GLOSSARY

GSI: gametophytic self-incompatibility
SC: self-compatibility
SI: self-incompatibility
SSI: sporophytic self-incompatibility