The population genetics of sporophytic self-incompatibility in *Senecio squalidus* L. (Asteraceae) I: *S* allele diversity in a natural population

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Twenty-six individuals of the sporophytic self-incompatible (SSI) weed, *Senecio squalidus* were crossed in a full diallel to determine the number and frequency of *S* alleles in an Oxford population. Incompatibility phenotypes were determined by fruit-set results and the mating patterns observed fitted a SSI model that allowed us to identify six *S* alleles. Standard population *S* allele number estimators were modified to deal with *S* allele data from a species with SSI. These modified estimators predicted a total number of approximately six *S* alleles for the entire Oxford population of *S. squalidus*. This estimate of *S* allele number is low compared to other estimates of *S* allele diversity in species with SSI. Low *S* allele diversity in *S. squalidus* is expected to have arisen as a consequence of a disturbed population history since its introduction and subsequent colonisation of the British Isles. Other features of the SSI system in *S. squalidus* were also investigated: (a) the strength of self-incompatibility response; (b) the nature of *S* allele dominance interactions; and (c) the relative frequencies of *S* phenotypes. These are discussed in view of the low *S* allele diversity estimates and the known population history of *S. squalidus*.
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Introduction

Senecio squalidus L. (Oxford Ragwort) is a short-lived perennial weed with a strong system of self-incompatibility (SI) (Abbott and Forbes, 1993). Like other members of the Asteraceae, SI in *S. squalidus* is sporophytic (Hiscock, 2000a, b). Self-incompatibility serves to prevent self-fertilisation and to restrict inbreeding amongst related plants, where incompatibility interactions are often controlled through the activity of a single *S* locus (reviewed in Hiscock and Kues, 1999). The *S* locus is a genetic region of suppressed recombination that appears to consist of many linked genes expressed in either maternal or paternal reproductive tissue with specific male and female gene products interacting to elicit the SI response that prevents fertilization (Hiscock, 2002). In SSI, the pollen apparently expresses both *S* alleles present in the parental sporophyte, whereas in the other common form of SI, gametophytic self-incompatibility (GSI), pollen grains express only one *S* allele encoded by the haploid, male (gametophyte) genome. These differences in the apparent expression of *S* alleles in pollen between GSI and SSI systems have important consequences for the reproductive and evolutionary dynamics of species that possess either SI system. In GSI systems, half-compatible phenotypes result from crosses between plants sharing one *S* allele,

whereas in SSI systems, such crosses are usually fully incompatible. Strict codominance between *S* alleles is necessary for functional GSI, whereas in SSI there are no restrictions on the evolution of complex dominance interactions between *S* alleles and dominance hierarchies between *S* alleles form an intrinsic part of most SSI systems (Hiscock and Kues, 1999). In SSI systems, dominance interactions allow for the natural occurrence of individuals homozygous for recessive *S* alleles, which are absent in normally functioning GSI systems (Stevens and Kay, 1989). In SI systems, mate availability (MA, defined as the average proportion of compatible mates in a population) is maximised and mating system equilibrium reached, at equal *S* phenotype frequencies (isoplethy) (Finney, 1952). For GSI systems, where dominance interactions between S alleles are absent, isoplethy is equivalent to equal *S* allele frequencies, whereas in SSI systems, isoplethy results in *S* allele frequencies that are dependent on the dominance interactions between the *S* alleles, such that the more recessive alleles occur at higher frequencies than dominant *S* alleles (Byers and Meagher, 1992; Schierup *et al*, 1997).

New *S* alleles, complementary sets of male and female *S* genes, are believed to evolve very rarely and only under limited selective scenarios, including temporary breakdown of SI, or through dual-specificity alleles (Matton *et al*, 1999; Uyenoyama *et al*, 2001). A fundamental consequence of SI is that the selective advantage of an *S* allele is inversely proportional to its frequency (negative frequency dependent selection) such that rare or new *S* alleles are favoured and maintained in popu-

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lations for extended periods of evolutionary time (Ioerger *et al*, 1990; Richman *et al*, 1996a). Negative frequency dependent selection results in the accumulation of *S* allele number despite the slow rate of *S* allele evolution, making the *S* locus one of the most polymorphic loci known, comparable with the MHC locus of mammals and the mating-type loci of fungi (Hiscock and Kues, 1999). An equilibrium balance will eventually be reached between the slow rate of evolution of new *S* alleles and their slow rate of loss. It is believed that the interaction between these evolutionary processes with particular SI systems and life history strategies will result in a characteristic species-specific *S* allele number (Richman *et al*, 1996a, b). Estimates of *S* allele number range from 15 to 66 for GSI species and from 33 to 49 S alleles for SSI species (Lawrence, 2000). *Trifolium* species represent a notable exception to these values, with estimated *S* allele number as high as 193. Unsurprisingly, species-level *S* allele estimates, other than for *Trifolium,* are not much higher than population-level *S* allele estimates (12 to 45 for GSI species, and 22 to 43 for SSI species, Lawrence, 2000) because inter-population migration and negative frequency dependent selection will effectively maintain numbers of *S* alleles in a population above that expected from individual population size alone (Wright, 1939).

In the British Isles, *S. squalidus* is commonly found in disturbed, urban habitats with well-drained soils and along roadsides and on railway lines. The introduction of *S. squalidus* and its subsequent spread in Britain has been well documented (Abbott, 1992; Harris, 2002). *S. squalidus* was first introduced into Britain, via the Duchess of Beaufort's Gardens at Badminton and the Oxford Botanic Garden, about 300 years ago from Mount Etna, Sicily (Harris, 2002). Following its escape from the Botanic Garden it became established in Britain in the early 19th century. From the mid-19th century onwards, the developing railway network provided the 'Oxford Ragwort' with many lanes of suitable habitat in the form of clinker beds and it went on to colonise successfully other British cities. Today, *S. squalidus* is found in most cities and towns throughout Britain, as far north as the Great Glen in Scotland, and also parts of Ireland (Harris, 2002). The recent history of introduction and colonisation by *S. squalidus* in Britain is likely to have caused a loss of genetic diversity and the establishment of appropriate selective pressures for a breakdown of its SI system. However, studies of SI in *S. squalidus* demonstrate quite clearly that it has a fully functional SSI system (Abbott and Forbes, 1993; Hiscock, 2000a, b).

Here we report the first detailed population genetic study of the mating system of *S. squalidus*. The aims of this study were to determine if there is any variation in the strength of SSI and to estimate *S* allele diversity in a typical British population of *S. squalidus*.

Materials and methods

Plants
Twenty-six *S. squalidus* seedlings (Ox1–Ox27, excl. Ox7) were collected from a wild population growing within a 0.5 km radius of Oxford railway station (grid ref.: SP505064) in March 1999. The seedlings were grown up and maintained in a glasshouse in 20 cm pots of soil-based potting compost with regulated watering and 16 h day lighting. When required, individuals were propagated by taking stem cuttings.

Pollinations
Fruit (achene)-set was chosen as a reliable measure of compatibility phenotype in *S. squalidus* because incompatible pollinations usually result in little or no fruit set, in comparison to up to 100 fruits set per capitulum for a compatible cross (Abbott and Forbes 1993; Hiscock, 2000a). Fruit set was recorded by counting the number of filled fruits produced per capitulum. Filled fruits, containing a fertilized ovule, appeared fatter and more pigmented than fruits containing an unfertilized ovule, which were thin and pale in colour. The strength of SI in each plant was tested by bagging capitula at the bud stage and leaving until the capitula had fully dehisced. Capitula were not emasculated prior to cross-pollination because these procedures caused excessive stigma damage. Instead, SI was relied upon to prevent the majority of illegitimate self fruit-set and an average of up to two fruits set per capitulum were scored as incompatible to allow for a low level of illegitimate self fruit-set. Indiscriminate cross-pollination was prevented by bagging capitula with pollination bags at the bud stage and re-bagging them with individual perforated paper bags after pollination (Hiscock, 2000a). Cross-pollinations were carried out by gently brushing flowering capitula together. Each cross was repeated reciprocally two to six times until a full cross diallel had been achieved. Average fruit-set per capitulum was calculated and compatibility phenotypes scored according to the scheme described in the key to Figure 1.

Construction and analysis of mating table diallel
A mating table diallel was constructed based upon the compatibility phenotypes of all 26 individuals and used to identify *S* alleles based on groupings of mutually incompatible individuals, similar to the methods described in Hiscock (2000a). It was reasoned that plants in such groups shared at least one dominantly expressed *S* allele resulting in the same *S* phenotype. A plant was chosen at random and all plants with which it shared an incompatible interaction were retained in its potential *S* allele-sharing pool. From this reduced set of plants, another was chosen at random, and the grouping process repeated until an incompatibility group was formed for which every member individual had been tested in this way. A plant not belonging to this incompatibility group was then chosen at random and further incompatibility groups formed until all plants had been assigned an *S* phenotype. *S* phenotypes represented by only one sample plant (ie Ox19) were considered valid if that plant had already been rejected from every other incompatibility group during the grouping process.

Estimating the number of *S* alleles in a population
Population *S* allele number was estimated according to the equations (equations 1 and 2) used previously to estimate *S* allele number in populations of GSI species. Because these estimators require *S* allele data to be in a diploid genotype format, equivalent to that which would be found under GSI conditions of strict *S* allele codominance, minimum and maximum numbers of *S* alleles in the sample were estimated by assuming that all unidentified *S* alleles were equivalent or that all unidentified *S*

		S1						S2			S3			S4										S5		S6
		1	5	9	11	16	24	2	3	25	4	12	20	6	8	13	14	15	18	21	22	23	26	17	27	19
S1	1	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	±	+	+	+	+	+	+	+	+
	5	-	-	-	-	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
	9	-	-	-	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
	11	-	-	-	-	-	-	+	+	+	+	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	16	-	±	±	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	24	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	-	+	+	+		+	+	+	+	+
S2	2	+	+	+	+	+	+	-	-	-	+	+	+	+	±	+	+	+		+	+	+	+	+	+	+
	3	+	+	+	+	+	+	-	-	-	+	+	±	+	-	+	+	+	+	+	+	±	±	+	+	+
	25	+	+	-	+	+	+	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
S3	4	+	+	+	+	+	+	+	+	+	-	-	-	±	+	+	+	+	+	+	+	+	+	+	+	+
	12	+	+	+	+	+	+	+	+	+	-	-	-	+	+	-	+	+	+	+	+	-	+	+	+	±
	20	+	+	+	+	+	+	+	+	±	-	-	-	+	+	+	+	+		+	+	+		+	+	+
S4	6	+	+	+	+	+	-	+	+	-	+	+	+	-	-	-	-	-	-	-	+	-	-	+	+	+
	8	+	+	+	+	+	+	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+
	13	+	+	+	+	+	+	+	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	+	+	+
	14	+	+	+	+	+	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+
	15	±	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-
	18	+	+	+	+	+	+	+	+	+	±	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+
	21	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+
	22	+	+	±	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+
	23	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-
	26	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+
S5	17	+	+	+	+	+	+	+	±	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
	27	+	+	-	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	±	-	-			+
S6	19	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-

Average fruits per capitulum	Classification when selfed	Classification when crossed	Symbol/Shading on diallel
0 – 2	self-incompatible	incompatible	-
>2 – <10	self-compatible	indeterminate	±
≥10	self compatible	compatible	+
cross not done	not classified	not classified	None

Figure 1 Analysed diallel of cross results between pairs of *Senecio squalidus* plants from an Oxford population. The first row and column refer to the dominantly-expressed *S* alleles that have been identified through analysis of this mating table (see Methods). The second row and column refer to named sample individuals with plants acting as pollen donor listed across the columns and plants acting as maternal receptor listed down the rows. The rest of the diallel consists of cross results for pairs of plants, classified and colour-coded according to the average fruit-set results described in the key.

alleles were unique. This provided a range of possible *S* allele number estimates for the population.

$$\frac{n}{m} = \frac{n}{N} \log\left(\frac{1}{1-\frac{n}{N}}\right) \qquad (1)$$

(Whitehouse, 1949)

$$n = N\left(1 - \left(1 - \frac{2}{N}\right)^r\right) \qquad (2)$$

(Paxman, 1963)

where *n* is the number of *S* alleles identified in a sample, *N* is the estimated number of *S* alleles present in the entire population, *r* is the number of plants sampled, and *m* is the number of *S* alleles sampled ($r = 2m$).

To avoid generating the artificial minimum and maximum *S* allele sample estimates required for the GSI estimators described above, we modified estimator equations 1 and 2 so that they could be used for SSI studies where dominance interactions between *S* alleles generally result in the identification of a single *S* allele per individual. These modified estimators are presented below as equations 3 and 4 (corresponding to modified versions of

equations 1 and 2, respectively). Equation 4 was originally derived from equation 2 by Lawrence (1996) to estimate population *S* allele number for the two locus GSI system found in the Poaceae, but may also be applied to the problem of estimating population *S* allele number for SSI systems. Equation 3 was derived from equation 1 using similar principles to those used by Lawrence (1996) to modify the Paxman (1963) estimator, namely that *m*, the number of *S* alleles sampled becomes, *r*, the number of plants sampled ($m = r$). Similarly, the error range for the modified Paxman (1963) estimator (equation 4) can be derived from the error range for the original estimator (equation 2) presented in Paxman (1963). The assumption of equal *S* genotype and *S* allele frequencies at mating system equilibrium is effectively replaced with the assumption of isoplethy at mating system equilibrium. The performance of the modified Paxman (1963) estimator relative to its unmodified counterpart was tested using Monte Carlo simulations of published diploid *S* genotype datasets and associated population *S* allele number estimates for natural SSI populations of other species (Stevens and Kay, 1989; Kowyama *et al*, 1994), where the modified estimator was calculated from 1000

resampled haploid datasets consisting of single randomly chosen S alleles.

$$\frac{n}{r} = \frac{n}{N} \log \left(\frac{1}{1 - \frac{n}{N}} \right) \tag{3}$$

$$n = N \left(1 - \left(1 - \frac{1}{N} \right)^r \right) \tag{4}$$
(Lawrence, 1996)

Measuring the thoroughness of the SI study

Repeatability (R) is a standard measure of the thoroughness with which an S allele assay has been carried out and was calculated according to equation 5. The resulting value is a proportion ranging from zero (as many different S alleles identified as S alleles sampled) to one (the minimum number of S alleles possible for a SSI system (ie, 2) identified in the entire sample).

$$R = 1 - \frac{n-2}{r-2} \tag{5}$$
(Stevens and Kay, 1989)

Testing the equality of S phenotype frequencies (isoplethy)

Since the dominantly expressed S alleles identified in our sample also correspond to the S phenotypes, a test of the prediction of isoplethy at mating system equilibrium was appropriate. Isoplethy was tested using the χ^2 test described in equation 6, which is itself a modified version of the χ^2 test developed for use in GSI studies (Campbell and Lawrence, 1981) so that it deals with single S allele genotype data.

$$\chi^2_{n-1} = \frac{n}{r} \left(\sum_{i=1}^{n} C_i^2 - \frac{r^2}{n} \right) \tag{6}$$
(Fearon *et al*, 1994)

where C_i is the frequency of occurrence of the ith S allele.

The significance of this χ^2 statistic was evaluated according to the Monte Carlo simulation methods described by Richman *et al* (1996b), using $r = 25$ and $n = 6$.

Measuring average mate availability for the sample

An average mate availability (MA) value for the sample was calculated according to equation 7. The resulting value is a proportion ranging from zero (all individuals inter-incompatible) to one (all individuals inter-compatible).

$$MA = \frac{1}{r} \sum_{i=1}^{r} \frac{r - C_i}{r - 1} \tag{7}$$
(Byers and Meagher, 1992)

Results

The strength of SSI in *S. squalidus*

Fruit-set counts from four to 20 selfed capitula per plant, confirmed that 25 out 26 plants in the Oxford population of *S. squalidus* were strongly SI (Table 1). One plant (Ox10) was classed as pseudo-self-compatible (PSC), according to Levin (1996), because its average fruit-set after selfing (13 fruits per capitulum, Table 1) was significantly higher than the self-fruit-set for all other plants

tested but lower than the average fruit-set after crossing. All fruit-set counts based on cross pollinations involving this plant demonstrated fairly high levels of fruit-set (data not presented) so that incompatible crosses could not be identified with any certainty, making it impossible to ascribe an S phenotype to this plant.

Identification of S alleles

Analysis of fruit-set counts from approximately 1800 cross-pollinated capitula in a complete diallel allowed the identification of six incompatibility groups or S phenotypes corresponding to six different, dominantly expressed S alleles in the Oxford population (Figure 1 and Table 2). This SSI model of complete dominance satisfactorily accounts for the majority of the crossing results (87.7%, Table 3). Assuming co-dominance of alleles S4 and S5 in the stigma of plant Ox27, explains a further 2.7% of the crossing results (Table 3). Thus, assuming a SSI model and the dominance scheme proposed, it is possible to identify and classify 26 out of a potential 50 S alleles within this sample of 25 SI plants.

S allele diversity

When S allele number estimators developed for GSI species (equations 1 and 2) were applied to our data for *S. squalidus*, a wide range of estimates of S allele number was obtained ranging from seven to more than 34 S alleles for the entire Oxford population. Modified estimators were therefore developed that could take into account the dominance relationships between S alleles that are characteristic of SSI (equations 3 and 4). These modified estimators predicted approximately six S alleles for the entire Oxford population (without the large range associated with the GSI estimators), which was the same as the number of S alleles identified in the sample. R (repeatability value) was calculated as a measure of the thoroughness of S allele sampling and found to be high (0.83, Table 4), indicating that much of the total S allele diversity within the Oxford population was likely to be represented within our sample of 26 individuals. Different S phenotypes were present at significantly unequal frequencies in the Oxford population when compared to the null hypothesis of isoplethy or equal S phenotype frequencies expected at mating system equilibrium (χ^2 test P value of 0.02, Table 4). Average mate availability, for sample individuals was high (0.78, Table 4), but likely to be depressed relative to that expected for the number of identified S alleles due to unequal S phenotype frequencies. A test of the performance of the modified Paxman (1963) estimator relative to its unmodified counterpart revealed that the modified estimator provided population S allele number estimates very similar to the estimates provided by unmodified estimators where complete diploid S genotype datasets were available (Table 5).

Discussion

Low S allele diversity is a feature of the SSI mating system of *S. squalidus* in Britain

Our estimate of approximately six S alleles in the Oxford population of *S. squalidus* is probably close to the true population S allele number for this population because the modified estimators we used (equations 3 and 4)

Table 1 Average self-fruit-set values, for sample individuals from an Oxford population of *Senecio squalidus*

Plant	Ox1	Ox2	Ox3	Ox4	Ox5	Ox6	Ox8	Ox9	Ox10	Ox11	Ox12	Ox13	Ox14	Ox15
Self-fruit-set value (SE)	0	0.1 (0.1)	0	0	0	0	0	0	13 (1.6)	0.4 (0.4)	0.1 (0.1)	0	0	0

Plant	Ox16	Ox17	Ox18	Ox19	Ox20	Ox21	Ox22	Ox23	Ox24	Ox25	Ox26	Ox27	All plants	All SI plants
Self-fruit-set value (SE)	0	0.1 (0.1)	0	0.6 (0.4)	0.5 (0.3)	0	0	0	0	0	0	0	1.4 (0.3)	0.1 (<0.1)

Ox10 was classified as pseudo-self-compatible due to its high self-fruit-set. Two average self-fruit-set values were calculated, one for all individuals to give an average self-fruit-set value for the Oxford population, and one for self-incompatible individuals only, excluding Ox10, to give a strictly SI self-fruit-set value.

Table 2 *S* allele designation for *Senecio squalidus* individuals in the Oxford sample, based on a sporophytic self-incompatible *S* allele dominance interpretation of the analysed diallel presented in Figure 1

S allele designation	*S1*	*S2*	*S3*	*S4*	*S5*	*S6*
Plant	Ox1	Ox2	Ox4	Ox6	Ox17	Ox19
	Ox5	Ox3	Ox12	Ox8	Ox27	
	Ox9	Ox25	Ox20	Ox13		
	Ox11			Ox14		
	Ox16			Ox15		
	Ox24			Ox18		
				Ox21		
				Ox22		
				Ox23		
				Ox26		
				Ox27(♀)		

Two *S* alleles could be assigned to plant Ox27 as its *S* alleles interacted in a partially codominant manner. ♀ associated with the *S4* allele of plant Ox27 refers to this *S* allele's codominance interaction with allele *S5* in maternal stigma tissue but recessiveness to *S5* in pollen.

Table 3 Summary statistics for cross-classifications and the explanatory power of the sporophytic self-incompatibility model of near-complete *S* allele dominance interactions for the analysed diallel of cross results in the Oxford *Senecio squalidus* population

Cross classifications	
Crosses classed as incompatible	27.1%
Crosses classed as compatible	70.6%
Undetermined crosses and missing datapoints	2.4%
Explanatory power of the fitted SSI model	
Crosses explained by designation of dominantly expressed *S* alleles	87.7%
Crosses explained by designation of tissue-specific codominance interactions between *S* alleles in plant Ox27	2.7%
Crosses not explained by *S* allele designations	9.6%

Table 4 Analysis of *S* allele diversity based upon *S* allele designations derived from a sporophytic-self-incompatible interpretation of classified cross results for an Oxford *Senecio squalidus* population. Equations 1–7 are described in the methods

Parameter	Estimator	Value (range of values)
Number of *S* alleles estimated to be in entire population (n)	Eq. 1	7.01–34.08
	Eq. 2	7.00–33.19
	Eq. 3	6.10
	Eq. 4	6.05–6.09
Thoroughness of study (R)	Eq. 5	0.83
χ^2 test of isoplethy	Eq. 6	13.16 ($P = 0.02$)
Average mate availability (MA)	Eq. 7	0.78

allowed for dominant *S* allele interactions of the type encountered in a species with SSI. The modified estimators permit the estimation of population-level *S* allele diversity without recourse to the minimum and maximum estimates of *S* allele number required by estimators used for GSI species (equations 1 and 2). GSI estimators unavoidably and greatly inflate the error bounds of the resulting estimates of population *S* allele number,

but the modified estimators rely on less stringent assumptions about *S* allele frequencies and distributions in populations. Also, the modified estimators merely assume equal *S* phenotype frequencies, including the possibility of *S* homozygotes, rather than assuming the equal *S* genotype frequencies and strict *S* heterozygosity required by the unmodified GSI estimators. Monte Carlo simulations of resampled diploid *S* genotype datasets for other SSI species (*Sinapis arvensis* and *Ipomoea trifida*) show that the modified Paxman (1963) estimator (equation 4) performs very well in comparison to its unmodified counterpart (equation 2) since the two estimators provide equivalent population *S* allele estimates, despite resampled haploid datasets being less informative than the original diploid datasets (Table 5). This is good corroborating evidence that the modified estimator has been appropriately applied and that the number of *S* alleles in the Oxford population of *S. squalidus* is close to the estimate of six *S* alleles provided by the modified estimators.

A population *S* allele estimate, equivalent to the sample estimate provided by the modified estimators but lower than the minimum estimate provided by the unmodified GSI estimator, is a consequence of no longer needing to assume the presence of a universal unidentified recessive *S* allele (ie, *S7*). As part of a normally functioning SSI system, all *S* alleles should have representative *S* phenotypes at isoplethy within populations such that the most recessive *S* allele in a dominance series will not only be present at high frequency but will also be expressed as an *S* phenotype class of its own, composed

Table 5 Results of Monte Carlo simulations to compare the performance of modified population *S* allele estimators relative to unmodified counterparts using 1000 haploid *S* genotype datasets resampled from previously published diploid *S* genotype datasets for natural populations of two SSI species *Sinapis arvensis* and *Ipomoea trifida*. Equations 2 and 4 are described in the methods

SSI species	Sample size (m)	Number of identified *S* alleles (n)	Population *S* allele number estimate (N; Eq. 2)	Population *S* allele number estimate (N_{mod}; Eq. 4) (S.E.)
Sinapis arvensis	35[a]	35[a]	43.24	40.56 (1.88)
Ipomoea trifida	40[b]	21[b]	21.42	18.47 (0.49)

[a]Individual *S* genotype scorings for a South Wales population (Stevens and Kay, 1989).
[b]Individual *S* genotype scorings for population G81 (Kowyama *et al*, 1994).

entirely of *S* homozygous individuals (Byers and Meagher, 1992, Schierup *et al* 1997).

The finding of non-isoplethy of *S* phenotypes (χ^2 test, $P = 0.02$, Table 4) is worth considering in the context of the assumptions of the modified estimators. In general, non-isoplethy results in underestimation of population *S* allele number, since some *S* alleles will be rare and less likely to be represented in samples (Campbell and Lawrence, 1981, Lawrence, 2000). Unequal *S* allele and *S* phenotype frequencies have previously been observed in natural populations of SSI *Sinapis arvensis* (Stevens and Kay, 1989) and GSI *Papaver rhoeas* (Campbell and Lawrence, 1981). Some of the possible reasons for non-isoplethy, which were thoroughly investigated in *P. rhoeas*, include: populations not being at mating system equilibrium; stochastic variation in frequencies at mating system equilibrium; and selection at loci linked to the *S* locus (Brooks *et al*, 1996; Lawrence, 2000). Non-isoplethy of *S* phenotypes in *S. squalidus* could also be attributable to either non-equilibrium mating system in highly anthropogenic *S. squalidus* habitats or stochastic variation in sampling frequencies due to such factors such as unequal plant size. However, since the absence of mating system equilibrium cannot be distinguished from the range of other causes of non-isoplethy without extensive further study of the samples, a population *S* allele estimator dependent on isoplethy is considered reasonable until more detailed knowledge of long term mating dynamics in *S. squalidus* becomes available.

The preference for an estimate of close to six *S* alleles in the Oxford *S. squalidus* population, based on the greater theoretical applicability of modified estimators rather than the original GSI estimators, is supported by other independent sources of evidence. The high proportion of incompatible crosses (27.1%, Table 3) in the cross diallel, provide an intuitive indication that many individuals in the sample share *S* alleles (Bateman, 1947). A similar proportion of incompatible crosses (34.5%) were found in a previous study of SI in British *S. squalidus* (Abbott and Forbes, 1993). The high repeatability value for the present study (R = 0.83, Table 4), corresponding to few *S* alleles identified relative to the number of *S* alleles sampled, suggests that 26 plants was probably enough to represent all the *S* allele diversity in the Oxford population. In a previous study of SSI in *S. squalidus*, Hiscock (2000a) identified as few as three *S* alleles, with one universal recessive *S* allele, in four *S. squalidus* individuals sampled from locations several kilometres apart in Oxford, based on crosses between force-selfed progeny arrays (Hiscock 2000a, b), which is further evidence that very few *S* alleles are present in the British population.

An estimate of six *S* alleles in *S. squalidus* is consider-

ably lower than previous estimates made for other SSI species, such as *Brassica campestris*, 22 and 31 *S* alleles for two different populations (Nou *et al*, 1993), and *Sinapis arvensis*, 43 *S* alleles for a single population (Stevens and Kay, 1989). Low *S* allele number in *S. squalidus* is probably a consequence of the extreme population bottleneck conditions predicted for the species during its introduction and establishment in the British Isles (Abbott and Forbes, 1993; Hiscock, 2000b). Unfortunately, important details about the introduction of *S. squalidus* such as the size of the initial founder population of *S. squalidus* introduced into the Oxford Botanic Garden and how plants were maintained and propagated before the 'escape' and subsequent dramatic population expansion during its spread away from Oxford are unknown (Harris, 2002). Nevertheless, detailed knowledge of the dynamics of the subsequent colonization of *S. squalidus* throughout Britain is available and further elucidation of this process is ongoing (Harris, personal communication).

Mating system consequences of low *S* allele diversity

Since *S. squalidus* appears to have fewer *S* alleles than are generally found at evolutionary mating system equilibrium for SSI, mate availability (MA) may restrict seed-set (Byers and Meagher, 1992). The fact that non-isoplethy of *S* phenotypes is a feature of the Oxford population does not appear to be reducing MA much further than low *S* allele number alone, since if the MA value calculated for the Oxford sample (77.6%, Table 4) is representative of the entire Oxford population of *S. squalidus*, then it is close to the deterministic MA value (83.3%) predicted for SSI populations consisting of six *S* alleles interacting to form a simple dominance series (Schierup *et al*, 1997). Furthermore, ecological factors such as small, variable population size, population substructure, or unequal reproductive capabilities, all of which are features of natural *S. squalidus* populations and which might serve to depress MA further, do not appear to be having a significant effect on reproductive assurance. The success of *S. squalidus* as a colonizing species in Britain suggests that low MA does not restrict the reproductive potential of *S. squalidus*. Other characteristics of the mating behaviour of *S. squalidus* may be sufficient to explain why sexual reproduction is not adversely affected by low MA in natural populations. The capitulum of *S. squalidus* consists of approximately 80–100 individual flowers with centripetal maturation over the course of about 1–2 weeks. This long flowering period for individual capitula under natural conditions makes multiple pollinator visits and fertilization opportunities a regular occurrence. Furthermore, each plant produces many capitula over the course of a long flowering season between April and

October (although, in Britain, sporadic flowering continues from November to March), so maximising the potential compatible mate pairing within a population for any given year. Mating potential is further optimized by the perennial habit (albeit short-lived) of *S. squalidus*. Nevertheless, despite these potential compensatory life history traits for low *S* allele diversity, the SSI system of *S. squalidus* itself is likely to have been subject to selective pressures to improve reproductive assurance since its introduction to Britain. Possible adaptive solutions to the problem of low *S* allele diversity include: (i) breakdown of SI leading to the evolution of self-compatibility (SC); (ii) evolution of new *S* alleles; (iii) introgression of *S* alleles into *S. squalidus* from other *Senecio* species; (iv) alteration of dominance interactions between *S* alleles; and (v) evolution at loci that modify SSI expression. We will now consider the potential contribution made by each of these evolutionary scenarios to the maintenance of SSI in *S. squalidus*.

(i) Breakdown of SI leading to the evolution of SC: Theory suggests that SC will evolve from SI whenever there is variation in the strength of expression of SI and the selective advantage of avoiding inbreeding depression under SI is less than twice the selective advantage of reproductive assurance under SC (Uyenoyama, 1986; Levin, 1996). Mutations that disrupt *S* allele activity, either directly in the form of self-sterility alleles or indirectly in the form of modifier loci causing pseudo-self-compatibility (PSC), are sufficient to provide variation in SI expression for selection to act upon. Population events that perturb the mating system and alter the selective balance between SI and SC, such as the purging of genetic load during population bottlenecks or the increased selective advantage of reproductive assurance during colonization may readily initiate a breakdown of SI (Levin, 1996). Within the Asteraceae, recent breakdown of SI has been documented in *Aster furcatus* as a result insufficient *S* allele diversity resulting from declining population size (Reinartz and Les, 1994). Even so, exceptions to this course of events are common and SI systems often survive intact through dramatic population events such as the introduction and spread of *Centaurea solstitialis* in western USA (Sun and Ritland, 1998) or the decline and fragmentation of *Hymenoxys acaulis* var. *glabra* populations (DeMauro, 1993). There is no evidence that SSI is breaking down in *S. squalidus* since British populations clearly maintain a strong, fully functional SSI system (Abbott and Forbes, 1994; Hiscock, 2000a, b, Brennan and Hiscock, unpublished). A simple model of SSI involving dominance and limited tissue-specific codominance between *S* alleles satisfactorily explains the majority of cross results (90.4%, Table 3) in our sample diallel, demonstrating that SSI in *S. squalidus* functions as expected. Most of the plants sampled from the wild were unambiguously SI when tested in the greenhouse (Table 1). Nevertheless, one PSC plant (Ox10), (average self-fruit-set = 13, Table 1) was identified in the Oxford sample and a low frequency of PSC plants can be found in other British populations of *S. squalidus* (1.33%, Brennan and Hiscock, unpublished). This may be evidence of selection acting to increase compatible matings, although the fact that the frequency of PSC is neither high nor approaching fixation implies that selection continues to favour the maintenance of SI over PSC or SC.

(ii) Evolution of new S alleles: The possibility that new *S* alleles may have evolved in British *S. squalidus* is unlikely given the relatively short period of time that has elapsed since its introduction to Britain, c. 300 years ago (approximately 300 generations). New *S* alleles are thought to evolve only under a limited range of evolutionary scenarios, such as the temporary evolution of dual specificity *S* alleles (Matton *et al*, 1999), or temporary breakdown of SI (Uyenoyama *et al*, 2001). Ancient *S* polymorphisms, often predating speciation, are typical of SI systems and further emphasise the rarity with which new *S* alleles arise (Ioerger *et al*, 1990; Richman *et al*, 1996a). The rate of evolution of new *S* alleles is responsive to increased selection pressures as indicated by accelerated rates of *S* allele diversification in *Physalis crassifolia* in response to population bottleneck events, but even this 'rapid' recovery of the SI system was on the scale of tens of thousands of generations post-population recovery (Richman *et al*, 1996b). *Senecio squalidus* provides a rare opportunity to test hypotheses on the short-term limits of *S* allele evolution by searching for unique 'British' *S* alleles in comparative surveys of *S* allele diversity in British and Sicilian populations.

(iii) Introgression of S alleles into S. squalidus from other Senecio species: It is possible that the success of *S. squalidus* as a colonizer in Britain may have been the result of introgression of new *S* alleles from other *Senecio* species during cultivation in the years since the introduction of *S. squalidus* to Britain, which could then account for the rapid spread of *S. squalidus* following a period of 150 years spent confined to the city walls of Oxford. This extra *S* allele diversity may then have been sufficient to alleviate any SI-related reproductive constraints and allow rapid range expansion beyond Oxford. There are no genetic barriers to hybridisation between *S. squalidus* and its putative parental taxa *S. chrysanthemifolius* and *S. aethnensis* and gene flow from these species into *S. squalidus* has been demonstrated by isozyme analyses of the three species on Mt. Etna, Sicily (Abbott *et al*, 2000). So much greater the potential then, for introgression of extra *S* alleles into *S. squalidus* if these and related *Senecio* species were cultivated together in the Duchess of Beaufort's gardens at Badminton or the Oxford Botanic Gardens (Harris, 2002). Hybridisation events between *S. squalidus* and native *Senecio* species, such as *S. vulgaris*, have also been recorded (Abbott, 1992). However, since *S. vulgaris* is self-compatible it is unlikely to have contributed extra *S* alleles directly to *S. squalidus* except possibly in the form of pseudogenes retained in the genome from an ancestral SI form. Certainly any newly introgressed *S* alleles would be subject to strong negative frequency dependent selection and likely to be maintained in populations even if the hybridizations were rare or resulted in hybrids of initially low fertility.

(iv) Alteration of dominance interactions between S alleles: The *S* alleles identified in the Oxford population sample demonstrated a high degree of dominance, with all but one of the sample plants (Ox27) expressing completely dominant *S* alleles with no evidence for the presence of fully codominant *S* alleles (Figure 1 and Table 2). This may be an adaptive feature of the SSI system in British populations of *S. squalidus* in response to low *S* allele diversity. The possibility of selectively altering domi-

nance interactions between *S* alleles in SSI systems provides an extra dimension of 'flexibility' to the SI system during times of population perturbation of the kind experienced by *S. squalidus*. In general, increased dominance between *S* alleles will result in increased MA relative to that predicted under a codominant SSI system (Byers and Meagher, 1992, Schierup *et al*, 1997).

This is a consequence of individual mating phenotypes being reduced to that of their dominant *S* allele alone, thereby increasing the MA by the proportion of plants in the population expressing the recessive *S* allele. Compatible crosses between close relatives, such as full sibs or parents and their progeny, become a regular feature of SSI in the presence of high levels of dominance between *S* alleles and have a pronounced effect on mating system dynamics. In particular, populations will experience a degree of inbreeding and its associated evolutionary implications (Uyenoyama, 1986), even in the presence of a fully functional SSI system. Allozyme studies have provided evidence for inbreeding in *S. squalidus*. Abbott *et al* (2000) calculated an F_{IS} (inbreeding coefficient) value as high as 0.22 but our allozyme data for the Oxford Population shows a lower but significant F_{IS} value of 0.03 (significantly greater than zero; $P < 0.01$; Brennan, unpublished data). Since, SI effectively limits selfing in the Oxford population (Table 1), inbreeding must be predominantly due to matings between close relatives where *S* allele dominance interactions allow.

Another potential benefit arising from increased compatibility between close relatives as a consequence of high levels of dominance between *S* alleles, is the potential improvement of a SSI species' colonizing ability during normal metapopulation dynamics. Single SI individuals still cannot found new populations (Pannell and Barrett, 1998), but as few as two *S* alleles in two or more compatible individuals will be sufficient to found a self-sustaining population (Schierup *et al*, 1997). Thus, increased dominance interactions between *S* alleles is a potential feature of SSI species that have experienced selection in response to colonisation. Further research to elucidate the particular dominance relationships between different *S* alleles in *S. squalidus* and comparison of these *S* allele interactions between British, Sicilian and other European populations may provide valuable insights into the evolution of *S* allele dominance interactions in SSI systems.

(v) Evolution at loci that modify SSI expression: Another evolutionary response of the SI system to selective pressures imposed by loss of *S* allele diversity could be evolutionary change at genetic loci that influence expression of SI – so called modifier loci, unlinked to the *S* locus (Levin, 1996). In general, these loci specifically suppress *S* allele expression resulting in SC, PSC or *S* phenotypes differing from those predicted by the genetics of the *S* locus alone and are hypothesised to provide SI systems with more flexible patterns of expression than are otherwise possible at a single genetic locus (Levin, 1996). PSC was observed in the offspring of some individuals of *S. squalidus* following forced selfing (Hiscock, 2000a, b) and its presence in natural populations has been explained as a consequence of selection for reproductive assurance during population bottlenecks or colonisation events (Levin, 1996). In SSI *Brassica* there is evidence that a cryptic system of GSI is operational and influences compati-

bility in certain genetic backgrounds and combinations of *S* alleles (see Lewis, 1994 for review). This cryptic GSI system is regulated by a single locus, *G*, with just two alleles, and generally leads to the occurrence of 'anomalous' compatibilities where the *S* allele phenotype would predict incompatibility. It is possible that a similar cryptic *G* gene system is also operational in *S. squalidus* and is responsible for the anomalous compatibilities observed in genetic analyses of SSI (Hiscock, 2000a, b). The activity of this locus might offer an explanation for some of the 9.6% 'anomalous' crosses identified in the diallel that remain unexplained by a sporophytic model of SI (Table 3).

Data presented here indicate that an Oxford population of *S. squalidus* contains approximately six *S* alleles. This population-level estimate of *S* allele number sets a new lower limit on the range of population *S* allele number estimates for species with SSI which previously ranged from 22 to 43 *S* alleles. It is argued that the low *S* allele estimates for *S. squalidus* may be a consequence of its unusual population history in the British Isles. The finding of non-isoplethy for *S* phenotype frequencies suggests that the Oxford population is not at mating system equilibrium and implies that high levels of disturbance continue to characterise British populations of *S. squalidus*. Low *S* allele diversity is likely to have significant consequences for mating dynamics, but SSI has clearly not broken down in British *S. squalidus* nor is it likely that extra, compensatory *S* allele diversity, in terms of *S* allele number, has evolved yet in British populations of *S. squalidus*. Other features of the SSI system of *S. squalidus* would seem more likely to account for the maintenance of a fully effective SI system with relatively few *S* alleles. These features include a high level of dominance observed between *S* alleles, the influence of modifier loci, and the reproductive life history of *S. squalidus*.

Acknowledgements

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