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ORIGINAL ARTICLE

The effect of sheltered load on reproduction in *Solanum carolinense*, a species with variable self-incompatibility

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Abstract In previous studies, we have investigated the strength of self-incompatibility (SI) in *Solanum carolinense*, a highly successful weed with a fully functional SI system that inhabits early successional and other disturbed habitats. We have found that the SI response in *S. carolinense* is a plastic trait—its strength being affected by the age of the flowers, and the presence of developing fruits and that there are genetic differences among families in their self-fertility. However, in species with a fully functional SI response, selfing would not be that common. As a result, deleterious recessives scattered though the genome of horsenettle are only occasionally exposed to selection. It has been suggested that deleterious recessives accumulate near *S*-alleles in strong SI species because the *S*-locus is located in a non-recombining region of the genome and because strong *S*-alleles are never in the homozygous state, thus sheltering some of the genetic load near the *S*-locus from selection. We performed a series of laboratory and greenhouse experiments to determine the extent to which sheltered load adds to the overall magnitude of inbreeding depression in horsenettle. Specifically, we amplified and sequenced the *S*-alleles from 16 genets collected from a large population in Pennsylvania and performed a series of controlled self-pollinations. We then grew the selfed progeny in the greenhouse; recorded various measures of growth and reproductive output; and amplified and sequenced their *S*-allele(s). We found that the heterozygous progeny of self-pollinations produce more flowers and have a greater ability to set both self and cross seed than *S*-homozygous progeny. We also found evidence of variation in the magnitude of load among *S*-alleles. These results suggest that sheltered load might slow the fixation of weak (partially compatible) *S*-alleles in this population, thus adding to the maintenance of a mixed mating system rather than leading to the fixation of the selfing alleles.

Keywords Breakdown in self-incompatibility · Inbreeding depression · Sheltered load · *Solanum*

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Introduction

The majority of flowering plants display both male and female reproductive structures in the same flower. Although this arrangement facilitates the deposition and collection of pollen by pollinators in just one visit, it also creates the potential for self-fertilization. Self-fertilization is problematic because it increases homozygosity, thereby reducing the contribution of overdominance to fitness and exposing deleterious recessives to selection. As a consequence, selfed progeny tend to suffer from inbreeding depression, i.e., the reduction in fitness of selfed offspring compared to outcrossed offspring (Charlesworth and Charlesworth 1987; Husband and Schemske 1996).

Because of its adverse effects on fitness, inbreeding depression has been regarded as a major force in the

evolution of plant mating systems (Darwin 1876; Barrett and Harder 1996; Barrett 2003). Many species of plants have evolved traits that reduce selfing. Such traits include the morphological positioning of the sex structures (herkogamy, enantiostily, Jesson et al. 2003), temporal uncoupling of maturation of the male and female parts within flowers (protandry and protogyny Bertin 1993; Sargent et al. 2006) and biochemical recognition and rejection of self-pollen (self-incompatibility Clark and Kao 1994; Kao and McCubbin 1996; Newbigin and Uyenoyama 2005).

Self-incompatibility (SI) is a genetic mechanism, controlled by a single highly polymorphic locus called the *S*-locus; each polymorphic variant is referred to as an *S*-allele (Lewis 1944; de Nettancourt 1977). SI allows a pistil to recognize and reject self-pollen prior to fertilization, based on biochemical interactions between pollen and pistils (McClure et al. 1990; Stone and Goring 2001; de Graaf et al. 2006). In the Solanaceae, SI disrupts the growth of pollen tubes that have an *S*-allele in common with the pistil they pollinate (Franklin-Tong and Franklin 2003; Kao and Tsukamoto 2004) thus avoiding fertilization. The disruption of self-pollen tube growth is caused by specific ribonucleases (called *S*-RNases) produced by the *S*-alleles in the pistil (McClure et al. 1989). These RNases enter the growing pollen tubes, where they degrade messenger and ribosomal RNA of pollen tubes identified as incompatible (Luu et al. 2000). This generalized degradation eventually arrests pollen tube growth (Wang et al. 2003; Kao and Tsukamoto 2004).

Solanum carolinense L. is a rhizomatous short-lived perennial, native to the eastern United States and Canada. *S. carolinense* has a fully functional gametophytic SI system, typical of the Solanaceae (Hardin et al. 1972; Richman et al. 1995). Unlike most self-incompatible plants, however, *S. carolinense* is a weed that inhabits early successional habitats, waste places, crop fields and pastures. It is listed as a noxious weed by the USDA and NRCS (2002) and the Seeds Act and Regulations of Canada (Basset and Munro 1986) and it is classified as an invasive weed in all of the 43 states in which it has been reported. Self-incompatibility is uncommon in weeds and early successional species (Baker 1955; Byers and Meagher 1992) because disturbed habitats require frequent episodes of colonization (hence populations are repeatedly founded by one or a few individuals bearing a limited number of *S*-alleles), effective population sizes are small (supporting few *S*-alleles, hence compatible cross pollen may limit fruit and seed production), and habitats are often short-lived (so there is limited time for the migration of additional *S*-alleles into populations).

In previous studies, we have investigated this apparent anomaly (i.e., a highly successful weed that is self-incompatible) and we have found that the SI response in *S. carolinense* is a plastic trait—its strength being affected by the age of the flowers (Stephenson et al. 2003), and prior reproductive success (Travers et al. 2004) and that there are genetic differences among families in their self-fertility (Mena-Alí 2006). In short, these studies reveal that when outcross pollen is scarce (older flowers remain unpollinated) and there is little or no outcross fruit set, some plants are capable of setting self seed. We have also recently shown that variability in self-fertility is associated with particular *S*-alleles (i.e., plants carrying certain alleles set significantly more selfed seed than plants not carrying these alleles, Mena-Alí et al. 2008). The importance of this variation in self-fertility on the ability of horsenettle to found and establish new populations will depend, to a large extent, on the magnitude of inbreeding depression.

As a species with a fully functional SI response, selfing in horsenettle is expected to be uncommon. As a result, deleterious recessives scattered though the genome of horsenettle are only occasionally exposed to selection. Furthermore, Uyenoyama (1997) hypothesized that deleterious recessives accumulate near *S*-alleles in strong SI species because the *S*-locus is located in a non-recombining region of the genome (Coleman and Kao 1992) and because strong *S*-alleles are never in the homozygous state. Consequently, some of the genetic load near the *S*-locus is even more sheltered from selection than unlinked deleterious recessives. In the study reported here we performed a series of laboratory and greenhouse experiments in order to determine the extent to which sheltered load adds to the overall magnitude of inbreeding depression in horsenettle.

Materials and methods

Plant material

Solanum carolinense is a weedy, herbaceous perennial that is found in ephemeral habitats and agricultural fields throughout southeastern Canada and central and eastern United States (Britton and Brown 1970). Once established it spreads via horizontal rhizomes that can extend over 1 m from the parent stem (Ilnicki et al. 1962), easing in the invasion and spread of newly colonized areas (Basset and Munro 1986). The above-ground parts die soon after the first frost in the autumn, marking the end of both the flowering and fruiting season. The below-ground parts overwinter and new shoots emerge early in the spring. Both growth and reproduction are indeterminate. The flowers are approx. 3 cm in diameter, with five partially fused white to violet petals; five stamens with short filaments and large yellow anthers (6–9 mm long) grow together and surround the exerted pistil. The flowers are

visited by pollen-gathering bees, which must vibrate the flowers to remove pollen from the poricidal anthers (Hardin et al. 1972). Inflorescences consist of 1–20 flowers that mature acropetally. The fruit is a globose berry, smooth and glabrous, yellow or orange at maturity, 10–20 mm in diameter, which typically contain 60–100 seeds (Basset and Munro 1986; Mena-Alí 2006). The majority of the flowers are perfect and functionally hermaphroditic. However, some of the flowers, usually located at the tip of the raceme, exhibit reduced non-functional pistils and are considered functionally staminate (Solomon 1985).

Horsenettle plants were collected from a large population located near State College, PA. Rhizome cuttings were taken from 20 plants that were at least 5 m. apart, in order to decrease the possibility of taking rhizomes from the same genet. These cuttings were brought to the greenhouse, planted in one-gallon pots, allowed to resprout, grow and flower. After flowering, we cut the stems off and moved the pots to a cold room set at 4°C to vernalize for 6–8 weeks. After the cold treatment, the pots were returned to the greenhouse and allowed to acclimate for a week. We then created ramets from each of the 20 genets (plants) by dividing the rhizome into 5–6 pieces of similar size. Each rhizome cutting was replanted in a one-gallon pot and allowed to resprout and grow. Four of the ramets were used in the controlled pollination experiment (see below), and the remaining ramets were returned to the coldroom. All of the ramets from two of the original 20 genets failed to resprout and therefore could not be used in this study.

Controlled pollinations on the parental generation

We divided the four ramets per genet into two groups. We performed only outcrossed pollinations on two ramets and only self-pollinations on the other two ramets. On both self-only ramets and both cross-only ramets per genet, we performed the assigned (i.e., self or outcross) pollinations every 3–4 day (flowers typically last 5–7 day in the greenhouse) on every flower that opened until a total of 40 flowers per ramet were pollinated. The outcrossed pollinations were performed by collecting pollen from at least five different genets using a buzz-pollination device (a modified electric toothbrush) in a microcentrifuge tube, vibrating the tube to thoroughly mix the pollen, and then touching the mixture to a stigma. Self-pollinations were made in the same manner except that pollen was collected from two to three flowers on the same plants as the flowers to be pollinated. At maturity (approx. 6 weeks) the fruits were collected and the number of mature seeds produced per fruit was recorded; the seeds were air-dried for 1–2 days and then stored in plastic vials with some desiccant. Two of the 18 genets used in this experiment did not produce enough flowers to complete all pollinations and were therefore excluded from this study. All 16 remaining genets produced at least 20 selfed seeds from the two selfed ramets combined.

Greenhouse experiments using selfed progeny

In order to determine the presence and the extent of sheltered load in *S. carolinense*, we used the progeny obtained from the controlled pollinations. For each of the 16 genets, we sowed 20 selfed seeds in plastic trays in the greenhouse and allowed them to germinate; we recorded the number of days to germination and the total number of seeds that germinated. After the first true pair of leaves was produced, we randomly selected six selfed seedlings per genet and planted them in one-gallon pots. These pots were distributed on greenhouse benches in a randomized block design, with one plant per genet in each block (for a total of six blocks). We recorded the number of days to first flower and the number of perfect and staminate flowers produced by each plant one day per week. Because flowers last 5–7 days in the greenhouse, these counts underestimate total flower production. Consequently, at the termination of flowering/fruiting we harvested the inflorescence and counted the number of flower scars. We also self-pollinated 5–7 flowers on each plant and allowed these flowers to set fruits. Two weeks later, we outcrossed 5–7 flowers on each plant. (We delayed the cross pollinations because our previous studies indicated that self fruits would not set if outcross fruits were already developing on a plants). At maturity, we collected the fruits and counted the number of seeds in each fruit. We calculated the index of self-compatibility (ISC) for (1) the number of fruits per pollination, and (2) the number of seeds per fruit using the formula$\mathrm{ISC} = n_{\mathrm{self}}/n_{\mathrm{outcrossed}}$, where n_{self} is the count obtained after self-pollinations and $n_{\mathrm{outcrossed}}$ is the count obtained after outcross pollinations; an ISC value of 1 indicates complete self-compatibility, whereas an ISC of 0 corresponds to complete self-incompatibility. In summary, we were able to calculate the ISC for the self progeny from each of the original 16 genets.

To determine if sheltered load associated with *S*-alleles contribute to inbreeding depression in horsenettle, we analyzed the data collected for this selfed progeny using a mixed model ANOVA with genet as a random effect and allele number (i.e., heterozygous or homozygous genotype) as a fixed effect. The measures of growth and reproduction included the days to first flower, the number of perfect, staminate and total flowers, the number of fruits per pollination, the number of seeds per fruit, the number of seeds per pollination and the values of ISC from fruit and seed set. All proportion variables were arcsine (square root) transformed prior to analysis.

S-allele genotype determination

In order to determine the *S*-genotype of the parental plants and their progeny, we used a modified PCR-based screening protocol, using allele-specific primers (Table 1, Lu 2006); a detailed description of the methods was presented by Mena-Alí and Stephenson (2007). Briefly, young leaves were collected in the greenhouse in liquid nitrogen, and stored at −80°C. Total genomic DNA was extracted from leaf tissue using Plant DNAzol (Invitrogen) and Ribonuclease A (Invitrogen) and resuspended in 50 µl of DEPC-treated water. Each plant was screened simultaneously for all *S*-alleles present in the parental population to ensure proper genotype determination and to reduce the possibility of false-positive amplification; selected parental genets comprising all *S*-alleles present in the original population were amplified along with the progeny samples in order to serve as positive controls. The PCR amplification of *S*-alleles was carried out in a 20 µl volume reaction containing 20 ng of DNA, 10× PCR buffer, 10 mM of each dNTP, 10 ng of each forward and reverse allele-specific primers, and 1 unit of HotStart *Taq* DNA polymerase. The reaction was incubated at 95°C for 3 min, followed by 30 cycles of 1 min at 95°C, 1:30 min at 60°C and 1:30 min at 72°C, and a final extension step of 5 min at 72°C. For allele S_{18} a touchdown protocol was used, with five cycles of 1 min at 95°C, 1:30 min at an initial annealing temperature of 60°C with a 1°C decrease per cycle and 1:30 min at 72°C, followed by 25 cycles of 1 min at 95°C, 1:30 min at 55°C and 1:30 min at 72°C and a final extension step of 5 min at 72°C. PCR products were run in a 1% agarose gel; positive products were cleaned with ExoSAP, and sent for sequencing at the Nucleic Acid Facility at PSU.

Results

Of the 96 self progeny (6 from each of 16 maternal plants), we were able to amplify and sequence the *S*-alleles from only 87; nine individuals died at different stages during the experiment and were omitted from the analyses. Of the 87 selfed progeny, 55 were homozygous at the *S*-locus and 32 were heterozygous (Table 2). A χ^2 test reveals that this excess of homozygous progeny differs significantly from the expected 1:1 ratio ($\chi^2 = 6.08$; $df = 1$; $P < 0.025$). The mixed effect analyses of variance revealed that the selfed progeny that were heterozygous at the *S*-locus produced significantly more flowers and had significantly greater seed set following self-pollinations than did selfed progeny that were homozygous at the *S*-locus (Table 3; Fig. 1).

Discussion

In a previous study (Mena-Alí et al. 2008) of *S. carolinense*, we found significant levels of inbreeding depression for flower, fruit and seed production per fruit and the vigor of the resprouts obtained from rhizome cuttings. The study reported here reveals that selfed progeny that are *S*-homozygotes have significantly higher levels of inbreeding depression for flower production than do selfed progeny that are *S*-heterozygotes (Fig. 1). These findings suggest the presence of sheltered genetic load (*sensu* Uyenoyama 1997) near the *S*-locus and they indicate that the sheltered load consists of genes with effects that are scattered over the lifespan of selfed individuals. Moreover, our data suggest that there is allele-specific sheltered load. When we compare the selfed progeny produced by the 12 S_8S_x maternal plants, the selfed progeny produced by the eight $S_{18}S_x$ maternal

Table 1 Allele-specific primers used in this study. Primer sequences first described/used by the following sources: (1) From Lu (2006); (2) From Mena-Alí and Stephenson (2007)

Primer name		Primer sequence (5′–3′)	Source	Fragment size (bp)		
Richman et al. (1995)	Lu (2006)			w/o intron	w/intron	intron
S1F	B+	cagcacgcaatgttgaatgac	(1)	142	261	119
S1R	B−	cataacgccagaaactttgtgt				
S5F	E+	agggtacactgctgcagga	(1)	134	211	77
S5R	E−	tgaaggttgtttcgccaagg				
S8F	H+	cagatataaagggcacagtgc	(1)	146	238	92
S8R	H−	ccagaaaccttgatttttccga				
S9F	J+	agagaaaagacgtctgcagtt	(1)	149	269	120
S9R	J−	tgtattgttcgtgccagagc				
S17F	R+	cgctgcttcagttctgtaag	(2)	312	400	88
S17R	R−	taactgtcttgacggcctc				
S18F	I+	gaattcayggnytntggccnga	(2)	149	257	108
S18R	I−	atgcccagaatgtttgatgctt				

Table 2 A compilation of the *S*-genotypes for 87 selfed progeny of *Solanum carolinense* used in this study, determined with allele-specific primers

Parental genets		Selfed progeny					
		S1	S2	S3	S4	S5	S6
1	S_5S_8	S_8S_8	S_8S_8	S_5S_8	S_8S_8	S_5S_5	S_5S_5
2	S_5S_{17}	S_5S_5	S_5S_{17}	S_5S_5	S_5S_5	–	S_5S_{17}
3	S_8S_9	S_8S_9	S_8S_9	S_8S_9	–	S_8S_9	S_8S_9
4	S_8S_9	S_9S_9	S_8S_9	S_8S_8	S_9S_9	S_8S_9	S_9S_9
5	S_8S_9	S_8S_8	S_8S_9	S_9S_9	S_9S_9	S_8S_9	S_8S_9
6	S_1S_{17}	–	–	S_1S_1	S_1S_1	S_1S_1	S_1S_1
7	S_8S_{18}	S_8S_{18}	S_8S_8	S_8S_{18}	S_8S_8	S_8S_{18}	S_8S_{18}
8	S_8S_{18}	S_8S_8	S_8S_8	S_8S_8	S_8S_8	S_8S_8	S_8S_8
9	S_8S_{18}	–	S_8S_{18}	$S_{18}S_{18}$	S_8S_{18}	–	S_8S_{18}
10	S_8S_{18}	S_8S_{18}	S_8S_8	S_8S_8	S_8S_{18}	S_8S_{18}	S_8S_8
11	S_8S_{18}	S_8S_8	S_8S_{18}	S_8S_8	S_8S_{18}	S_8S_{18}	S_8S_8
12	S_8S_{18}	S_8S_8	S_8S_{18}	S_8S_8	S_8S_{18}	S_8S_8	S_8S_8
13	S_1S_5	S_5S_5	S_5S_5	S_1S_1	S_1S_1	S_1S_1	S_5S_5
14	S_8S_{18}	–	S_8S_8	S_8S_{18}	S_8S_{18}	S_8S_{18}	S_8S_8
15	S_1S_{18}	$S_{18}S_{18}$	S_1S_1	$S_{18}S_{18}$	–	–	S_1S_1
16	S_1S_8	S_8S_8	S_8S_8	S_8S_8	S_8S_8	S_1S_8	S_8S_8

Table 3 Mixed model analysis of variance for several vegetative and reproductive traits, between homozygous and heterozygous selfed progeny grown under greenhouse conditions

Dependent variable	Effect	*df*	*F*	*P*
Days to flower	Genet	15, 69	2.26	0.0118
	HetHom	1, 69	0.35	0.5582
Staminate flowers	Genet	15, 69	1.77	0.0568
	HetHom	1, 69	0.11	0.7436
Perfect flowers	Genet	15, 69	1.74	0.0621
	HetHom	1, 69	3.24	0.0760
Total number of flowers	Genet	15, 69	1.79	0.0530
	HetHom	1, 69	3.89	0.0526
Outcross fruit per pollination	Genet	15, 69	0.67	0.8031
	HetHom	1, 69	0.57	0.4530
Outcross seed per fruit	Genet	15, 69	1.20	0.2925
	HetHom	1, 69	0.61	0.4375
Outcross seed per pollination	Genet	15, 69	1.23	0.2722
	HetHom	1, 69	0.77	0.3841
Self fruit per pollination	Genet	15, 69	3.33	0.0003
	HetHom	1, 69	2.76	0.1012
Self seed per fruit	Genet	15, 32	1.40	0.2051
	HetHom	1, 32	2.85	0.1011
Self seed per pollination	Genet	15, 69	2.85	0.0017
	HetHom	1, 69	8.19	0.0056
ISC (fruit)	Genet	15, 69	3.51	0.0002
	HetHom	1, 69	3.52	0.0648
ISC (seed)	Genet	15, 69	4.08	<0.0001
	HetHom	1, 69	5.49	0.0220

plants (the two most frequent of the five *S*-alleles found in the maternal plants; Table 2) and the selfed progeny produced by the three S_9S_x maternal plants (the allele that we had previously found to be the most self-fertile allele in our study; Mena-Alí and Stephenson 2007), we found that the S_{18} allele had lower levels of sheltered load, as evidenced by the significantly higher number of flowers in $S_{18}S_{18}$ compared to S_xS_x siblings (Table 4; Fig. 2). In contrast, allele S_8 had similar levels of sheltered load as its S_xS_x siblings (although S_8S_8 plants showed a slight increase of flowers produced as compared to S_xS_x siblings). Interestingly, homozygotes of the leaky S_9 allele produced significantly (28%) fewer flowers than their S_xS_x siblings, suggesting that sheltered load may play a role in preventing the fixation of this allele by counteracting the reproductive assurance benefits of this allele (see below).

The presence of sheltered load in *S. carolinense* has been previously examined by Stone (2004). In her study, sheltered load was inferred by the significant deficiency of homozygous progeny following bud pollinations (self-pollinations performed before anthesis and before the *S*-RNases accumulate in the style). The deviation was shown to be the result of seed abortion caused presumably by deleterious mutations linked to the *S*-locus. Our study extends her findings to later stages of the life cycle. Unlike Stone's (2004) study, however, we found an excess of *S*-homozygotes in our selfed progeny, a finding that is inconsistent with the sheltered load hypothesis. In fact, the preponderance of *S*-homozygotes among our selfed progeny raises the possibility that homozygotes of some *S*-alleles may actually be more fit than *S*-heterozygotes during early stages of seed development (although this initial advantage reverses in latter stages of growth and reproduction).

To investigate the over-abundance of *S*-homozygotes in more detail, we examined the progeny produced by the 12 maternal plants that had the S_8 allele and the progeny produced by the eight maternal plants that had the S_{18} allele (Table 2). Assuming random fertilization and no selection on the resulting seeds, S_xS_8 plants should produce progeny in a $1S_xS_x::2S_xS_8::1S_8S_8$ ratio following self-pollination. However, the selfed progeny from S_xS_8 plants reveal an excess of S_8S_8 homozygotes ($8S_xS_x::30S_xS_8::30S_8S_8$; $\chi^2 = 15.2$; $df = 2$; $P \ll 0.001$); this deviation suggests that S_8 alleles are more likely to self fertilize than other S_x alleles in the population. Since this ability does not segregate tightly with S_8 (Mena-Alí and Stephenson 2007) the increased ability to self fertilize is most likely be due to a reduced stability of the S_8-RNase. This is in line with our previous studies, which have suggested that plants with the S_8 allele possess a moderate ability to self fertilize

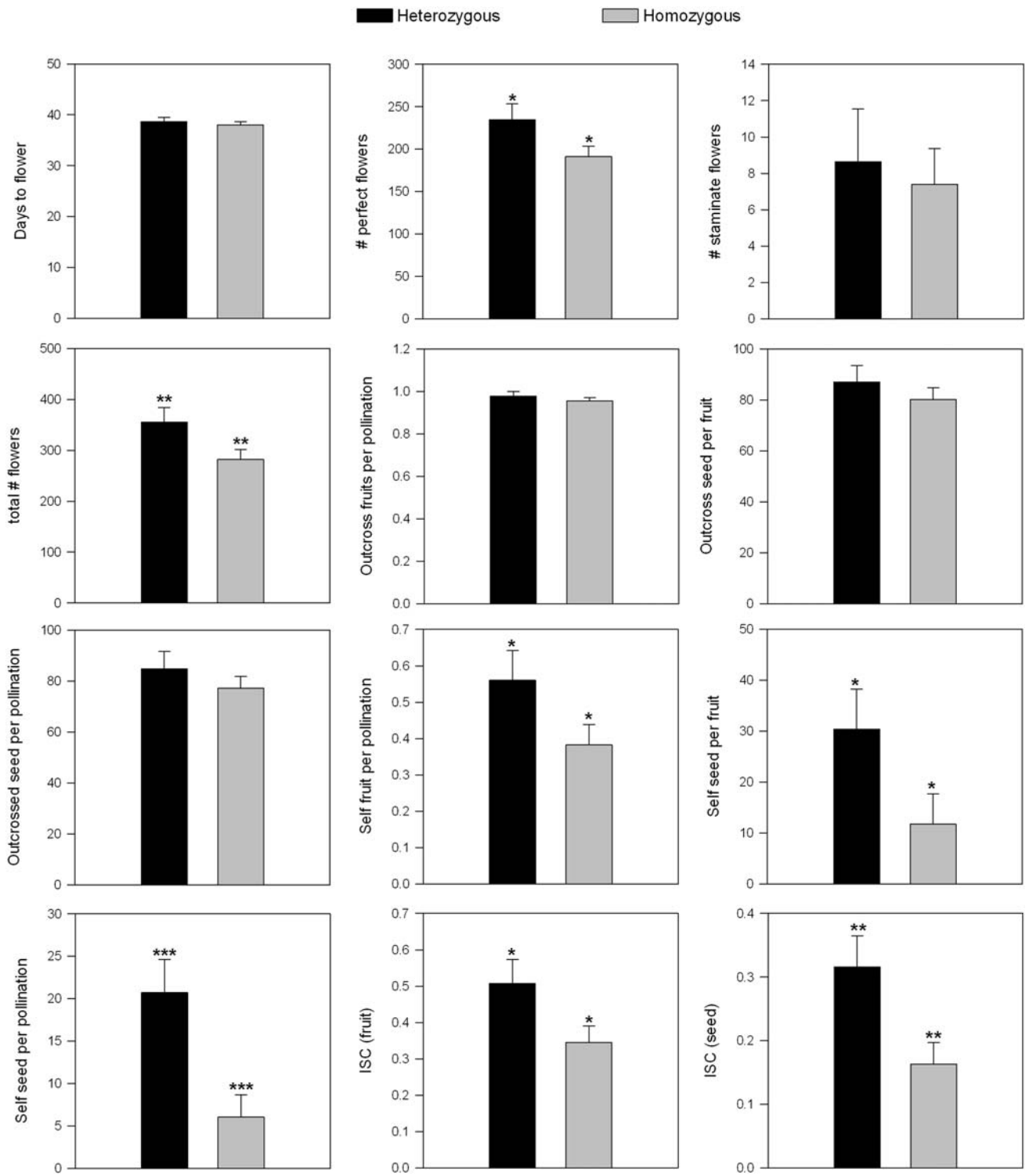

Fig. 1 Comparison of the means ± SE for 12 variables of growth and reproduction between heterozygous and homozygous selfed progeny as an indication of sheltered load associated with *S*-alleles of *Solanum carolinense*. Levels of significance indicated by *asterisks*: * $P < 0.10$; ** $P < 0.05$; *** $P < 0.01$

(Mena-Alí and Stephenson 2007). Moreover the preponderance of selfed progeny possessing the S_8 allele (the most abundant allele in parental plants and more likely to be transmitted via pollen to selfed progeny than an S_x allele) would at least partially account for the increased ability of homozygous progeny to self-fertilize.

Table 4 Mixed model analysis of variance for total flower production between homozygous and heterozygous selfed progeny bearing particular *S*-alleles as a measure of allele-specific sheltered load

	Effect	*df*	*F*	*P*
Total number of flowers	S8 genotypes	2, 84	0.96	0.3853
	S9 genotypes	2, 84	3.47	0.0357
	S18 genotypes	2, 84	3.29	0.0423

In contrast, the selfed progeny from S_xS_{18} plants show a deficiency in both the S_{18} homozygotes and the S_{18} heterozygous progeny (22 S_xS_x::18 S_xS_{18}::3 $S_{18}S_{18}$). There are two possible reasonable explanations for this deviation: pollen bearing the S_{18} allele is less likely to fertilize an ovule than S_x (i.e., S_{18} is strongly self-incompatible and the S_{18} in the progeny are inherited mostly via the ovule), or S_{18} is linked to a nearly lethal ovule/embryo/endosperm gene (the S_{18} allele is inherited mostly via the pollen). In this study, S_{18} was paired with S_8 in seven of the eight maternal plants possessing S_{18}. Therefore, it is likely that S_{18} is more strongly SI than the S_8 allele and is less likely than S_8 to be transmitted via pollen upon self-pollination. However, when we examined six outcrossed progeny produced by each of the eight S_xS_{18} maternal plants in this study (see Mena-Alí and Stephenson 2007) we expected to find a 1:1 ratio of S_S_{18} and S_S_x. Instead we found 19 S_S_{18} and 29 S_S_x (i.e., there is a nearly significant deficiency in S_S_{18} individuals among outcross progeny from S_xS_{18} moms) indicating that the transmission of S_{18} via ovules may be impaired. This combination of a weak SI allele (S_8), impaired transmission of S_{18} via the ovules, and a sample of plants skewed toward S_8S_{18} (7 of 16) could account for much of the overabundance of homozygotes and an under-representation of heterozygous selfed progeny. In short, we do not know the precise cause of the overabundance of *S*-homozygotes in our study but we suspect that *S*-homozygotes are not more fit during the initial stages of seed development. Rather, we suspect that there are differences among alleles in the strength of SI, the presence of an ovule/early seed lethal, some other factor, or some combination of factors) that skew the homozygote to heterozygote ratio.

Because various floral traits (including size and number of flowers, length of inflorescence) have been shown to be tightly linked to the *S*-locus in the genus *Solanum* (Bernacchi and Tanksley 1997) expression of *S*-locus-linked sheltered load is expected to directly affect these traits. If these traits are also tightly linked to the *S*-locus in *S. carolinense*, they provide a possible explanation for the presence of detectable sheltered load acting on later stages of the growth and reproduction. It should be noted that sheltered load, unlike unlinked deleterious recessives,

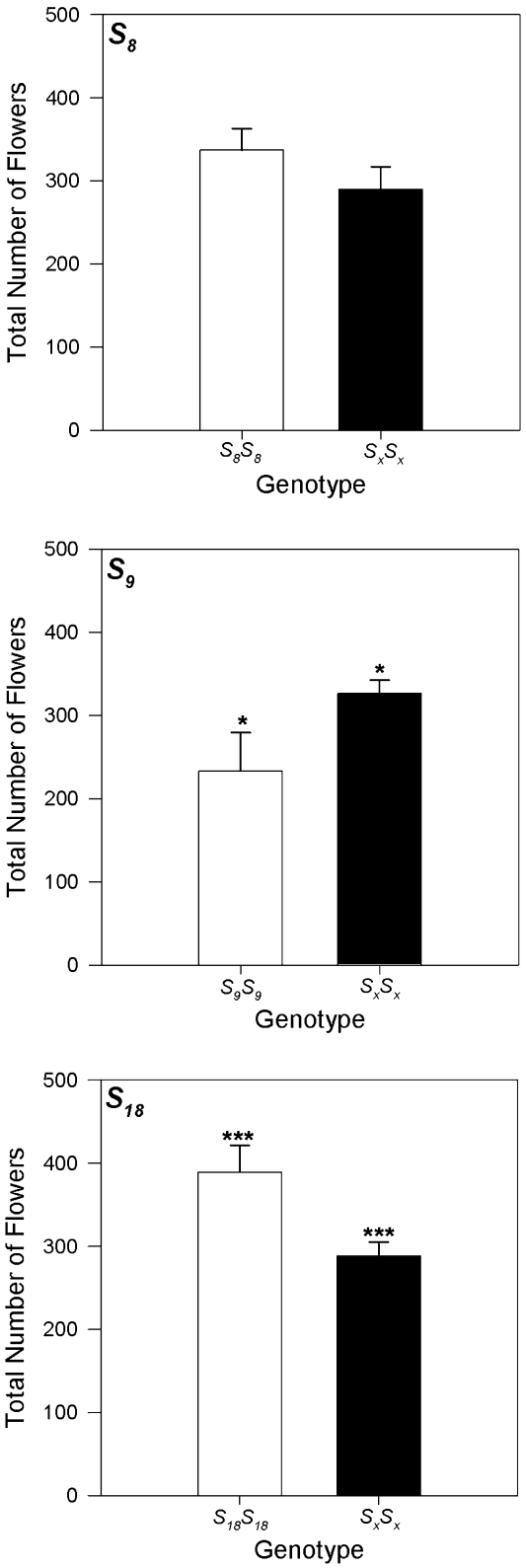

Fig. 2 Comparison of the means ± SE for total number of flowers produced between homozygous selfed progeny bearing particular *S*-alleles as an estimation of allele-specific sheltered load. Levels of significance indicated by *asterisks*: * $P < 0.10$; ** $P < 0.05$; *** $P < 0.01$

would be very difficult to purge even in successive generations of selfing. Consequently, the presence of sheltered load affecting both growth and reproduction in selfed plants would slow the fixation of selfing *S*-alleles in this population, thus adding to the maintenance of a mixed mating system rather than leading to the fixation of the *S*-alleles with enhanced self-fertility.

The ability to self-fertilize may be particularly important for predominantly outcrossing species that live in ephemeral habitats, occur in small populations, and experience frequent episodes of colonization and extinction, such as island colonizers and weeds (e.g., Baker 1955). Consequently, genetic variants that promote self-fertilization should increase in frequency unless they are opposed by other evolutionary forces such as inbreeding depression and pollen discounting (e.g., Nagylaki 1976; Holsinger et al. 1984; Charlesworth and Charlesworth 1987). The effect of such a genetic variant on the breeding system is ultimately determined by the balance in the different forces favoring (e.g., transmission advantage and reproductive assurance) and opposing (e.g., inbreeding depression) self-fertilization. It should be noted that horsenettle can also spread vegetatively via rhizomes and that a close association between clonality and self-incompatibility has recently been described for several species of *Solanum* (Vallejo-Marín and O'Brien 2007). This association between SI and clonal growth further supports the argument that vegetative reproduction may not only provide an alternative mechanism for persistence of *S. carolinense* in habitats where cross pollen limits reproduction but it can also influence the population structure and may ultimately favor the evolution of leaky alleles in weedy self-incompatible species. Because our study only examined two generations of controlled crosses, we were unable to tease apart the nature of the genes involved in the reduction in fitness associated with sheltered load. However, an examination of the progeny resulting from multiple-generations could provide us with a more detailed pattern of segregation and thus enable us to better understanding the complex interactions of genetic factors that determine the reproductive success of individuals of this weedy species under varying conditions.

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