ORIGINAL ARTICLE

DOES FREQUENCY-DEPENDENT SELECTION WITH COMPLEX DOMINANCE INTERACTIONS ACCURATELY PREDICT ALLELIC FREQUENCIES AT THE SELF-INCOMPATIBILITY LOCUS IN *ARABIDOPSIS HALLERI*?

Violane Llaurens,[1] Sylvain Billiard,[1] Jean-Baptiste Leducq,[1] Vincent Castric,[1] Etienne Karl Klein,[2] and Xavier Vekemans[1,3]

[1]*Laboratoire Génétique et Evolution des Populations Végétales, UMR 8016, CNRS, Université Lille 1, F-59655 Villeneuve d'Ascq France*

[2]*UR546, Unité de Biostatistique et Processus Spatiaux, INRA, Domaine St-Paul - Site Agroparc, F-84914 Avignon, France*

[3]*E-mail: xavier.vekemans@univ-lille1.fr*

Frequency-dependent selection is a major force determining the evolutionary dynamics of alleles at the self-incompatibility locus (S-locus) in flowering plants. We introduce a general method using numerical simulations to test several alternative models of frequency-dependent selection on S-locus data from sporophytic systems, taking into account both genetic drift and observed patterns of dominance interactions among S-locus haplotypes (S-haplotypes). Using a molecular typing method, we estimated S-haplotype frequencies in a sample of 322 adult plants and of 245 offspring obtained from seeds sampled on 22 maternal plants, collected in a single population of *Arabidopsis halleri* (Brassicaceae). We found eight different S-haplotypes and characterized their dominance interactions by controlled pollinations. We then compared the likelihood of different models of frequency-dependent selection: we found that the observed haplotype frequencies and observed frequency changes in one generation best fitted a model with (1) the observed dominance interactions and (2) no pollen limitation. Overall, our population genetic models of frequency-dependent selection, including patterns of dominance interactions among S-haplotypes and genetic drift, can reliably predict polymorphism at the S-locus. We discuss how these approaches allow detecting additional processes influencing the evolutionary dynamics of the S-locus, such as purifying selection on linked loci.

KEY WORDS: Balancing selection, mating system, S-locus, sporophytic self-incompatibility.

Self-incompatibility (SI) is a widespread genetic system that prevents selfing in hermaphrodite plant species (de Nettancourt 2001). SI phenotypes are generally controlled by a single genetic factor, the S-locus, which exhibits a high level of allelic polymorphism that Wright (1939) suggested is maintained by strong negative frequency-dependent selection (NFDS). Theoretical studies on SI have generally assumed that all alleles at the S-locus are selectively equivalent, meaning that the selection act-

ing on a given allele depends only on its frequency. All alleles are then expected to reach identical frequency at equilibrium in an infinite population (Nagylaki 1975; Boucher 1993).

However, recent theoretical (Uyenoyama 2003) and empirical (Bechsgaard et al. 2004; Chookajorn et al. 2004; Stone 2004) studies suggested that additional selective forces may act along with NFDS. First, purifying selection may be acting on sets of deleterious alleles segregating at loci linked to the S-locus, the

so-called "sheltered load" (Uyenoyama 2005). Linked deleterious alleles are indeed expected to be maintained because of the high heterozygosity, if recombination rates are low in the S-locus genomic region (Uyenoyama 1997, 2003). Variation among alleles at the S-locus (S-haplotypes) for the occurrence of such sheltered load and substantial distorted segregation in the gametes at the S-locus have indeed been reported in experimental studies of self-incompatible plant species (Bechsgaard et al. 2004; Stone 2004). Second, there may be variation in the strength and specificity of the pollen–pistil protein recognition (e.g., in *Brassica*, Chookajorn et al. 2004). The S-locus includes two distinct but tightly linked genes expressing two highly coadapted proteins interacting specifically with each other (Takayama and Isogai 2003). We thus named "S-haplotype" a given combination of linked alleles at the genes controlling male and female SI phenotypes. Chookajorn et al. (2004) suggested that S-haplotypes may vary in the strength and specificity of the pollen–pistil protein recognition. Such processes could be responsible for observed patterns of nonrandom loss of S-haplotype lineages during a strong bottleneck in the history of the genus *Physalis* (Richman and Kohn 1999).

Some evidence of NFDS acting on the S-locus exists for gametophytic SI systems in plants (Lawrence 2000). In gametophytic SI, phenotypes are determined gametophytically in pollen, whereas codominance occurs among alleles expressed in pistils. To verify the prediction of identical frequency among S-haplotypes, several authors have directly compared haplotype frequency data from natural population samples against the analytical expectation (reviewed in Castric and Vekemans 2004). In most species with gametophytic SI, the observed haplotype frequencies fitted the analytical theory, but in some plant species, strong departures were consistently reported. These were interpreted as resulting from nonequilibrium effects (Kato and Mukai 2004), or from selection at linked loci (Lane and Lawrence 1995). These analyses are flawed for several reasons, notably the fact that data from natural finite populations were used to test predictions derived assuming an infinite population, thus only considering sampling variance and neglecting drift variance, whereas the variance in haplotype frequencies will also depend on the relative strength of NFDS and genetic drift (Stoeckel et al. 2008). In Wright's original model of NFDS in plants with gametophytic SI, the strength of selection is strictly determined by the number of S-haplotypes maintained in the population (Wright 1939). However, this model assumed selection acting on male fitness only, and the inclusion of an additional component of NFDS acting on female fitness, through an increase in seed production for individuals expressing a rare SI phenotype could, under ecological conditions of pollen limitation, substantially increase the selection strength (Vekemans et al. 1998).

Additional complications arise in plants with sporophytic SI, where phenotypes at the S-locus are determined sporophytically in pollen, and complex patterns of dominance interactions occur among S-haplotypes (Bateman 1952). In particular, S-haplotypes may be ranked according to a linear dominance hierarchy, or belong to a number of discrete dominance classes, with codominance among haplotypes of the same class (Prigoda et al. 2005). Moreover dominance interactions may differ in pollen and pistils (Stevens and Kay 1989). Dominance in sporophytic SI introduces heterogeneity in effective selection coefficients among S-haplotypes; highly recessive haplotypes will be hidden from selection in heterozygotes, whereas the most dominant S-haplotypes are always exposed to selection (Schierup et al. 1997; Billiard et al. 2007). Recessive S-haplotypes thus reach higher deterministic equilibrium frequencies than more dominant ones. In a finite population, recessive S-haplotypes will be more prone to genetic drift, and thus exhibit higher variance around the deterministic equilibrium frequency than dominant ones. With dominance classes, the higher the dominance level, the higher the number of haplotypes expected to be maintained within the class, with a single haplotype in the most recessive class (Uyenoyama 2000; Billiard et al. 2007).

Detailed empirical studies on polymorphism and dominance patterns at the S-locus in natural populations with sporophytic SI are scarce. Molecular genotyping methods are needed to accurately estimate S-haplotype frequencies, but the genes involved in pollen–stigma recognition have only been identified in a single family with sporophytic SI (Brassicaceae). Also determining dominance interactions among S-haplotypes requires considerable numbers of pollination tests among genotypes. In a single population of *Arabidopsis lyrata* from Iceland, Mable et al. (2003) identified at least 11 S-haplotypes in a sample of 20 individuals and found that the haplotype with the highest frequency was also the most recessive, in agreement with theory. Prigoda et al. (2005) showed that S-haplotypes belong to discrete classes that correspond to phylogenetic clusters of alleles in an allelic genealogy at the pistil-specificity gene *SRK*, and found identical patterns of dominance interactions in pollen and pistil. Using these patterns of dominance and newly identified S-haplotypes in the same Icelandic population, Schierup et al. (2006) estimated the haplotype frequencies and found a close fit to the deterministic expectations. However, they did not take into account drift in their theoretical analysis, and stochastic simulations based on their data concluded that this population is certainly not isolated or not at equilibrium (Billiard 2008). In *Brassica insularis*, S-haplotypes belong to two molecular categories, assumed to correspond to two dominance classes (Nasrallah 1991), and Glémin et al. (2005) showed that S-haplotypes from the putative dominant class (class I) are more numerous and have lower frequencies than those from the putative

recessive class (Class II). However, some uncertainties in the dominance level of some S-haplotypes precluded more formal tests against theoretical models.

In this article, we investigate diversity at the S-locus in a small isolated natural population of *Arabidopsis halleri*, a species with fully functional sporophytic SI (Llaurens et al. 2008). More than 20 S-haplotypes have been identified in this species based on a sequence survey at the *SRK* gene (Castric and Vekemans 2007). We genotyped the S-locus in individuals from two successive generations and characterized dominance of the observed S-haplotypes using controlled pollinations. We then implemented a likelihood-based procedure using simulations to test the fit of several alternative models of NFDS to the S-haplotype frequency data, taking into account both genetic drift and the observed dominance interactions.

Materials and Methods
SPECIES STUDIED

Arabidopsis halleri (Brassicaceae) is a diploid perennial herb with sporophytic SI that is closely related to the self-incompatible *A. lyrata* and the autogamous *A. thaliana* (Clauss and Koch 2006). It is a European species growing in mountainous areas and on heavy-metal polluted sites, for example close to industrial areas.

POPULATION SAMPLING AND DNA EXTRACTION

We focused on a single isolated population located in Nivelle (France, 50°28′N, 3°27′E). This population was estimated to contain at least 800 individuals. Samples were collected as described in Llaurens et al. (2008). Briefly, we sampled $N = 322$ individuals collecting leaves from ramets that appeared to be distinct individuals. We also sampled seeds from 22 maternal plants ($N = 245$ from 110 fruits) and grew these offspring for eight weeks in a greenhouse. All leaf samples were oven-dried at 55°C, and DNA was extracted using the Nucleospin Multi-96 Plant extraction kit (Macherey-Nagel Gmbh, Düren, Germany).

SCREENING FOR S-HAPLOTYPES

We used a molecular genotyping method to screen for S-haplotypes using primers targeting the pollen-recognition extracellular domain of the S-locus pistil-expressed *SRK* gene (Takayama and Isogai 2005). In a previous study, 22 S-haplotypes (named AhS01 to AhS22 in the present study) were identified in *A. halleri*, based on a sequence survey at the *SRK* gene (corresponding, respectively, to *SRK* alleles AhSRK01 to AhSRK22, GenBank accession numbers EU075124–EU075143) in a European population sample (Castric and Vekemans 2007). Three additional S-haplotypes (AhS23, AhS24, and AhS26) have subsequently been identified (V. Llaurens and V. Ruggiero, unpubl. results). We used PCR primer pairs specific for each *SRK* allele (Table 1) to genotype individuals at the S-locus, generating

presence/absence data for each known S-haplotype. The reaction mixture (15μL) contained 20 ng DNA, 1X buffer (Applied Biosystems, Foster City, CA), 2 or 2.5 mM MgCl$_2$ (depending on the primers pair), 200 μM Fermentas dNTP mix (Fermentas Canada, Burlington, ON, Canada), 200 μg/mL BSA, 0.2 μM of each microsatellite primer, and 0.025 U/μL *Taq* polymerase (Amplitaq DNA polymerase, Applied Biosystems). PCR amplifications were performed with an MJ research PTC 200 thermocycler (MJ Research, Waltham, MA) with the following profile: 5 min at 95°C, 35 cycles of 40 sec at 95°C, 40 sec at annealing temperature (specific for each pair of primers, see Table 1), 40 sec at 72°C, and one cycle of 10 min at 72°C. PCR products were mixed with loading dye and run at 110 V on 2% agarose gels in TBE buffer for 45 min. Fragments were visualized by ethidium bromide under UV light and compared to a 100 bp DNA ladder. A positive control for each targeted S-allele was run on each gel. Overall, we screened the 322 parents and 245 offspring for presence/absence of each of the 25 S-haplotypes.

Five individuals were found to show three different S-haplotypes. No allele was consistently associated with these five genotypes, and the occurrence of polyploid individuals could be discarded based on preliminary results from a survey of DNA content variation within European populations of *A. halleri* (M. Pauwels, pers. comm.) and from observed patterns of segregation of 11 microsatellite markers in this population (Llaurens et al. 2008), so we hypothesized that this observation was probably caused by mixing of plant material at the time of sampling or to DNA contamination problems. We therefore discarded these individuals from further analyses.

ESTIMATION OF S-HAPLOTYPE FREQUENCIES

The genotyping method by presence/absence does not unambiguously assess whether individuals are homozygotes or heterozygotes with another unidentified S-haplotype. Because recessive S-haplotypes are expected to occur frequently as homozygotes in the population (Billiard et al. 2007), we assumed that individuals in which a single S-haplotype was identified were homozygous if this haplotype was recessive or with intermediate dominance. Otherwise, if the haplotype was dominant, individuals were considered heterozygous with an as yet undetected haplotype.

DOMINANCE INTERACTIONS AMONG S-HAPLOTYPES

We also performed controlled pollinations between individuals with known genotypes, using 35 plants from the offspring population. Pollinations were performed under a binocular magnifying glass. An anther of the chosen male partner was collected with tweezers and pollen was deposited on the pistil of the chosen female partner, within two days of flower opening, to limit pollen contamination. Pistils were marked individually and pistil length was measured seven days after pollination, a period sufficient for

Table 1. Primer sequences and PCR conditions to amplify S-haplotypes in *A.halleri*.

SRK allele	Targeted S-haplotype		5′ Primer sequence 3′	Annealing temperature	[MgCl$_2$] in PCR mix
AhSRK01	AhS01	F	TCAAGATTGAAGCTGAGTGA	57°C	2.5 mM
		R	TACACAACCCGTCCCGCCAA		
AhSRK02	AhS02	F	GGGTTATTACAGCGATTTGA	53°C	2.5 mM
		R	GCCCACTCTTGCGGGTACGG		
AhSRK03	AhS03	F	TGATATTTACATGGGGTGCA	51°C	2.5 mM
		R	CCTCTTGTCCACAATAGCAC		
AhSRK04	AhS04	F	GACAATAAATACCGTAGACG	52°C	2.5 mM
		R	ACGCCGTACAGTTACAATGT		
AhSRK05	AhS05	F	GATTTCAAAAGAACAGGATC	53°C	2.5 mM
		R	AATCCACATCAAACAACCAA		
AhSRK06	AhS06	F	AAGGGTTTCAACCTCTGTAT	51°C	2.5 mM
		R	TACAATTGCAATTCTCCCTA		
AhSRK07	AhS07	F	AGGATTGGGATTTTGGATTT	53°C	2.5 mM
		R	TCCTTACATTGTTCCAAAGA		
AhSRK09	AhS09	F	GAGTCACCACTCCCAATGTG	54°C	2.5 mM
		R	TCAGTGTTAGTGTTATCTAG		
AhSRK10	AhS10	F	GAGAATAGGGAGGAGGTCTG	56°C	2.5 mM
		R	CACCGCAATTCAATTGCCTA		
AhSRK11	AhS11	F	CAACGGAGATGGATTTCACA	53°C	2.5 mM
		R	GTCCTACCATCCCGGGTAAT		
AhSRK12	AhS12	F	ATCATGGCAGTGGAACACAG	58°C	2 mM
		R	CAAATCAGACAACCCGACCC		
AhSRK13	AhS13	F	GAGAAGATTGGAACCAACAT	51°C	2 mM
		R	CGTCTTCCTCACACACCCTT		
AhSRK14	AhS14	F	TTATACTTTCCGACTAATTA	50°C	2.5 mM
		R	CAGCCTTAGTAGCAGGAGAT		
AhSRK15	AhS15	F	ATTCCAACAGAACGTGAAAAT	55°C	2.5 mM
		R	ATCTGACATAAAGATCTTGACC		
AhSRK16	AhS16	F	GATTGGTATTCTGGAGATTG	53°C	2.5 mM
		R	CCTTCACATCAATTATTCTA		
AhSRK17	AhS17	F	GAGAATAGAGAGGAGGTCAT	57°C	2.5 mM
		R	GTACAAACCCTTTGATGCAT		
AhSRK18	AhS18	F	CGTGTGATATGTATGAAGAA	50°C	2.5 mM
		R	CTTTTTCCTTGCAGCTCAAG		
AhSRK19	AhS19	F	ATGTTCTGGCCTTCATCGGC	51°C	2 mM
		R	ATCCGGCACCTTCATTCTAC		
AhSRK20	AhS20	F	ACGTTCCTAATGACCAATTC	56°C	2.5 mM
		R	CTTTCACACCAATTCTCCGG		
AhSRK21	AhS21	F	CATCTACTCGAGATTGACCC	55°C	2.5 mM
		R	ATAACTCTTTGGTCCAGAAA		
AhSRK22	AhS22	F	ATTACATGGTTTACAACTTT	50°C	2.5 mM
		R	CCACTCCTGCTGGTTCTTGG		
AhSRK23	AhS23	F	CTGGGTTTCATCGGTACGCG	56°C	2.5 mM
		R	ACATAACTCCGAATATCGGT		
AhSRK24	AhS24	F	CAATGAGTTCCACAGGTGCT	56°C	2.5 mM
		R	ACCTCTTTTTGCATTCTCCA		
AhSRK26	AhS26	F	AACGGACCAGTGTGATATGA	56°C	2.5 mM
		R	GTCTTTCTCATCAATTTCCA		

total fruit elongation. For each pair of individuals, we performed at least 10 replicates of controlled pollinations in both directions, that is, each plant was used either as pollen donor or as pollen receptor. Pollination was classified as "successful" when the fruit was at least 6.1 mm long and contained developed seeds. A preliminary survey using plants with known phenotypes showed that crosses between plants with identical phenotypes did not exceed a fruit-length of 6.1 mm (J. B. Leducq, unpubl. data). We considered a pair of individuals as "compatible" in a given direction when "successful" pollination occurred in at least 50% of the replicates as in previous studies in *A. lyrata* (Prigoda et al. 2005).

If two heterozygous individuals sharing only one given S-haplotype were incompatible, we inferred that the shared S-haplotype was expressed in both pollen and pistil SI phenotype, thus excluding recessivity of the S-haplotype in both SI phenotypes. By testing each S-haplotype in a variety of genotypes, we characterized the dominance of most S-haplotypes both in pollen and pistil. In some cases, we used plants with homozygous genotypes, obtained by controlled crosses among plants with one S-haplotype in common.

COMPARING OBSERVED VERSUS EXPECTED DISTRIBUTIONS OF S-HAPLOTYPE FREQUENCIES

We simulated a finite panmictic population with sporophytic SI to generate expectations for the distribution of S-haplotype frequencies in random samples, with parameters chosen to mimic the empirical study. These simulations were performed with the software NESSI (Billiard 2008), which implements a general model of sporophytic SI (Billiard et al. 2007). The life cycle we assumed includes pollination and fertilization determining the genotypic frequencies at the S-locus (assuming that every individual produces an infinite number of pollen and ovules), followed by a random sampling of N individuals among all seeds produced during fertilization, to generate the next generation. This sampling follows a multinomial distribution, with the genotypic frequencies as parameters.

To test for the influence of dominance interactions among S-haplotypes, we simulated either strict codominance among haplotypes, or the empirically determined interactions. We also compared two different selection regimes, the so-called "fecundity selection model" where NFDS occurs through both male and female fitnesses (Vekemans et al. 1998), representing pollen limitation, and "Wright's model," where NFDS occurs through male fitness (Wright 1939). In cases in which dominance interactions could not be fully resolved with the pollination tests performed, we ran separate predictions for several alternatives.

We simulated a population of size $N = 800$, using the k-alleles model of mutations (with $k = 8$) and a mutation rate of $\mu = 10^{-6}$ (see Billiard 2008 for further details). After reaching approximate mutation–drift equilibrium of S-haplotypes (5000

generation), samples of size n were drawn at 1000 generations intervals for 100,000 generations, and each run was replicated 100 times. We recorded the numbers of S-haplotypes and their frequencies. For each S-haplotype, the distribution of frequencies over the 10,000 replicate samples, after discarding runs with loss of some S-haplotypes, was used to compute a 95% confidence interval for the mean frequency. The probability of loss of each S-haplotype was also estimated.

LIKELIHOOD COMPUTATIONS

To check the expectations obtained from simulations under different models and hypotheses against the empirical results, we estimated the likelihoods L of the observed allelic frequencies. For each simulation run x, we took account of sampling by computing the likelihood of the observed haplotype frequencies given the frequencies in the population, using the multivariate hypergeometric distribution for random draws without replacement:

$$L_x = \prod_{i=1}^{a} \binom{m_{x,i}}{k_i} / \binom{2N}{2n}$$ where N and n are as described above, $m_{x,i}$ is the number of copies of haplotype i among the $2N$ chromosomes in run x, k_i is the number of copies of haplotype i in the observed sample, and a the total number of haplotypes. The full likelihood including random sampling and genetic drift, defined as the expectation $\hat{L} = E[L_x]$ was computed as the average L_x, conditional on the existence of L_x, in other words when $m_{x,i} \geq k_i$.

To test whether different models fit the data significantly differently, we computed the $\triangle$AIC between two models as twice the difference between their log-likelihoods (no parameters are fitted). Although not providing a strict statistical test, $\triangle$AIC was considered "significant" and "highly significant" at values of 5 and 10, respectively (Anderson and Burnham 2002).

PREDICTING CHANGES IN S-HAPLOTYPE FREQUENCIES BETWEEN GENERATIONS

We used an ad hoc simulation framework to compute the frequency change expected between two successive generations. As previously, we simulated several alternative scenarios: (1) no SI system (model with drift only), (2) sporophytic SI with codominance among all S-haplotypes; (3) sporophytic SI with the observed dominance interactions and "Wright's selection" regime; (4) sporophytic SI with the observed dominance interactions and the "fecundity selection" regime. As initial conditions for the simulations, we used the observed genotypic composition of the adult (parental) population. We restricted mothers to those from which offspring had been collected, weighting by the number of offspring sampled from each mother. For pollen genotypic frequencies, we used the whole adult population. We performed 10,000 simulations, and computed for each S-haplotype the range of frequencies in the offspring corresponding to 95% of the simulations, discarding the simulation runs in which at least one haplotype was lost.

Results under each of the four scenarios were compared to the empirical results as follows. We computed the likelihood of the haplotype frequency data among the offspring as $L_y = \prod_{i=1}^{a} p_{y,i}^{k_i}$ with $p_{y,i}$ the expected frequency of haplotype i in offspring under scenario y, k_i the observed number of copies of haplotype i in offspring, and a the total number of S-haplotypes.

Results

S-HAPLOTYPE IDENTIFICATION

In the Nivelle population, we identified eight of the 25 currently known *A. halleri* S-haplotypes (AhS01, AhS02, AhS04, AhS12, AhS15, AhS20, AhS22, AhS24, Table 2). We unambiguously characterized heterozygous genotypes for 334 individuals of 567 (adult and offspring individuals) (Table 2). For 200 individuals, we identified only one S-haplotype and we assumed that 143 were homozygotes, as they carried the most recessive S-haplotypes (see below). Overall, 33 of 567 (5.8%) of all individuals produced no amplification with any of the primers. Because microsatellite alleles amplified in all individuals (Llaurens et al. 2008), this is unlikely to be due to poor DNA quality. Hence, we hypothesize that these individuals carry S-haplotypes that are not currently known. Overall, we obtained genotypic information for 295 individuals in the adult population sample and this number was used as the sample size n in the simulations and the likelihood computations.

DOMINANCE INTERACTIONS AMONG S-HAPLOTYPES

Figure 1 shows the proportion of successful pollinations among individuals with known genotypes at the S-locus. For most genotypes, self-pollination and pollinations with plants carrying an identical genotype were performed. According to our criterion (see Methods), these crosses were all incompatible, confirming the determination of the SI genotype, and showing that the SI system was functional. Crosses between different genotypes allowed characterization of the dominance interactions among S-haplotypes. Crosses between AhS01/AhS01 homozygotes and heterozygous individuals carrying only one copy of AhS01 were always compatible. We thus inferred that haplotype AhS01 is recessive with respect to every other S-haplotype tested. We then used crosses of different heterozygous genotypes with heterozygotes carrying AhS01, to test the dominance relationships among the other S-haplotypes. The dominance interactions among AhS01, AhS02, AhS04, AhS12, and AhS15 were fully determined. The dominance levels of S-haplotypes in pollen and pistils were correlated overall. However dominance in pollen was strictly hierarchical, whereas there are three main dominance classes in pistils, with codominance within classes and full dominance between classes (Fig. 2). In pistils, the most recessive class is a

Table 2. Occurrence of known *SRK* alleles in the adult and offspring populations of *A. halleri*. Number of individuals with 0, 1, 2, or 3 *SRK* alleles detected, and number of individuals carrying each allele.

	Number of individuals with											
	0 allele	1 allele	2 alleles	3 alleles	AhSRK01	AhSRK02	AhSRK04	AhSRK12	AhSRK15	AhSRK20	AhSRK22	AhSRK24
Adult sample (n=322)	27	129	166	5	124	38	55	97	36	95	23	8
Offspring sample (n=245)	6	71	168	0	94	27	75	72	35	75	15	13

	Maternal plants																Inferred paternal phenotype
GENOTYPES	AhS01/AhS01	AhS01/AhS02	AhS01/AhS04	AhS01/AhS12	AhS01/AhS15	AhS01/AhS20	AhS01/AhS22	AhS02/AhS02	AhS02/AhS04	AhS02/AhS12	AhS02/AhS15	AhS04/AhS12	AhS04/AhS20	AhS12/AhS15	AhS12/AhS20	AhS20/AhS24	
AhS01/AhS01	0.07 (0.05)	1.00	1.00	1.00	1.00	1.00	1.00										AhS1
AhS01/AhS02	0.83	0.0 (0.0)		1.00				0.00	0.10	0.20	1.00						AhS2
AhS01/AhS04	1.00		0.0 (0.0)			1.00			0.20			0.00	0.32			1.00	AhS4
AhS01/AhS12	1.00	1.00		0.0 (-)		0.67	1.00			0.00		0.00		0.00	0.20	1.00	AhS12
AhS01/AhS15	1.00				0.0 (0.17)						0.00			0.00			AhS15
AhS01/AhS20	0.90					0.0 (-)	1.00						0.06		0.20	0.20	AhS20
AhS01/AhS22	1.00						0.0 (-)										AhS22
AhS02/AhS02		0.00					1.00	0.0 (0.0)		0.00						1.00	AhS2
AhS02/AhS04		1.00	0.10						0.0 (0.0)								AhS4
AhS02/AhS12		1.00		0.00				1.00		0.0 (-)		0.00					AhS12
AhS02/AhS15		1.00			0.00						0.17 (0.0)			0.00			AhS15
AhS04/AhS12			0.80	0.12						0.25		0.0 (-)					AhS12
AhS04/AhS20			0.97			0.00							0 (0.21)				AhS20
AhS12/AhS15				1.00	0.00		0.80					0.00		0.0 (0.13)			AhS15
AhS12/AhS20				1.00		0.10									0.2 (-)		AhS20
AhS20/AhS24						0.00	1.00									0.1 (-)	AhS20 or AhS20/AhS24
Inferred maternal phenotype	AhS1	AhS2	AhS4	AhS12	AhS15	AhS20	AhS22	AhS2	AhS2/AhS4	AhS2/AhS12	AhS15	AhS4/AhS12	AhS20	AhS12/AhS15	AhS12/AhS20	AhS20 or AhS20/AhS24	

Figure 1. Proportions of successful pollination. Genotypes were considered as incompatible (gray cells) when less than 50% of the replicate pollinations were successful. Values along the diagonal represent results from self-pollinations and outcross pollinations with identical genotype (under parentheses). SI phenotype was inferred by observing the compatibility patterns among the crosses: contribution of each S-haplotype of the considered genotype to the phenotype was tested by crosses with plants with various genotypic combinations containing the tested haplotype.

single haplotype (AhS01), two haplotypes (AhS02 and AhS04) belonged to the second most recessive class, and two haplotypes (AhS15 and AhS20) belonged to the most dominant class. In pistils, haplotype AhS12 was codominant with haplotypes AhS02 and AhS04, but also with AhS15 and AhS20, which belong to two different dominance classes. Dominance of AhS20 in pollen was only partially determined because not all plants carrying the nec-

essary genotypic combinations could be generated. We were able to assess that AhS20 was dominant over AhS01, AhS02, AhS04, and AhS12 in pollen, but the relationship with AhS15 remains undefined. In pistil, AhS20 was codominant with AhS12 and AhS15. For two S-haplotypes, AhS22 and AhS24, we obtained few results because of missing genotypes. In both pollen and pistil, AhS24 was either recessive or codominant with respect to AhS20, and
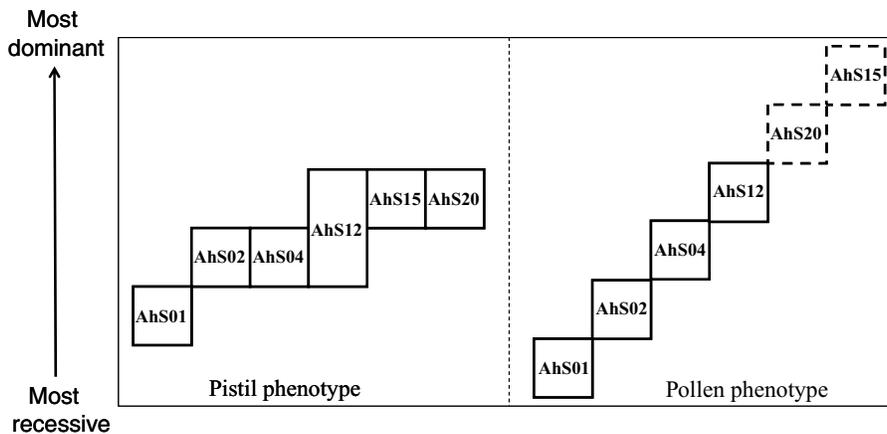

Figure 2. Dominance interactions among S-haplotypes in pollen and pistils, inferred from controlled pollinations in *A. halleri*. S-haplotypes are represented by squares whose vertical position indicates relative dominance level. Codominant alleles are represented side by side. Haplotype AhS12 is codominant in pistil with all other alleles except AhS01. Relative dominance of AhS15 versus AhS20 in pollen is undetermined.
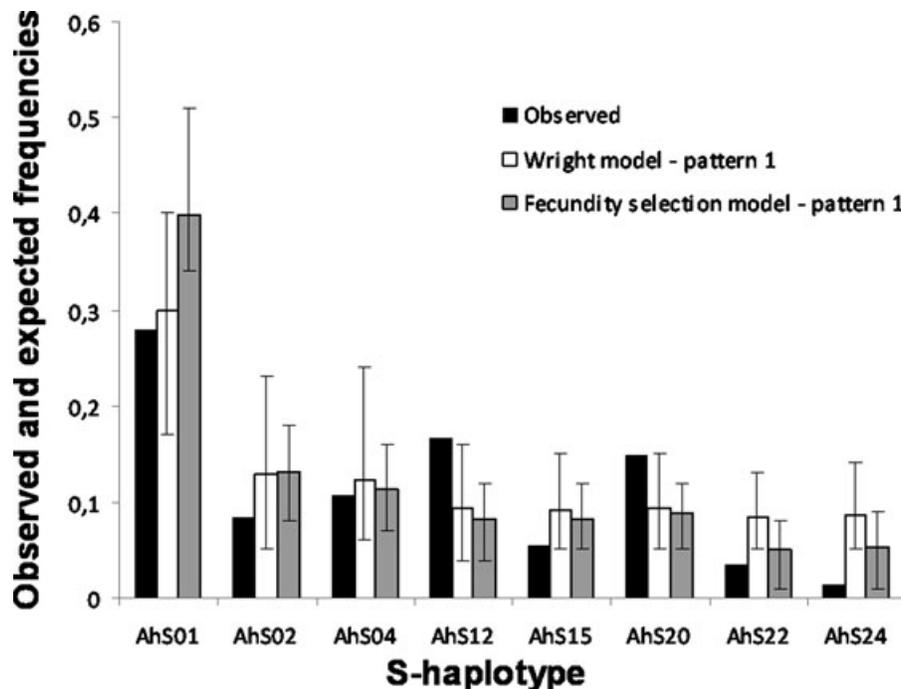
Figure 3. Frequencies of S-haplotypes observed in the adult population and predicted from finite population simulations according to dominance pattern 1 assuming three dominance classes in pistils and strictly hierarchical relationships in pollen (see Fig. 4). Black bars: observed frequencies in adult population; white bars: expected frequencies under Wright's model; gray bars: expected frequencies under the fecundity selection model; error bars represent the 95% confidence intervals obtained across 10,000 simulation runs.

AhS22 was dominant over AhS01. Overall, these results demonstrate for six S-haplotypes (all but AhS22 and AhS24) that they are expressed and functional in *A. halleri*.

COMPARING OBSERVED AND EXPECTED HAPLOTYPE FREQUENCIES IN THE ADULT POPULATION

When computing S-allele frequencies in the adult population sample, we assumed that when each of the most recessive S-haplotypes AhS01, AhS02, and AhS04 were found alone in an individual, this individual was a homozygote. The results showed that AhS01, the most recessive S-haplotype, had by far the highest observed frequency, as expected from theory (Fig. 3). In contrast, for the other haplotypes, we found no clear relationship between frequencies and dominance levels.

To simulate the evolution of haplotype frequencies at the S-locus, we used the dominance interactions inferred from the experimental pollinations, and for the unresolved cases (see above) we tested three alternative dominance patterns (Fig. 4). Under both frequency-dependent selection models (Wright's and fecundity selection models), pattern 1 provided the best fit to the empirical data, followed closely by pattern 2, whereas the worst fit was obtained when assuming strict codominance among all S-haplotypes (Table 3). This analysis demonstrates that the pattern of dominance interactions among S-haplotypes has a paramount influence on haplotype frequencies in natural populations. Wright's model under patterns of dominance 1 and 2 significantly outperformed

(ΔAIC > 25) the fecundity selection model (Table 3, Fig. 3). The rank orders of likelihoods of the different models and dominance patterns were consistent under various mutation rates μ and population sizes N (results not shown).

HAPLOTYPE DIVERSITY AT THE S-LOCUS

To determine whether haplotype diversity at the S-locus in this population was compatible with a model of mutation/drift/NFDS, we recorded the proportion of simulation runs in which any of the eight S-haplotypes was lost (Table 4). Both models of selection showed similar overall proportions of haplotype loss, with only 21% and 24% of simulations losing at least one S-haplotype, under Wright's model and the fecundity selection model, respectively. However, the distributions of allele losses were strikingly different between models. In Wright's model, only recessive S-haplotypes (AhS01, AhS02, or AhS04) were likely to be lost, whereas dominant S-haplotypes (AhS22 and AhS24) were more susceptible to drift in the fecundity selection model. This suggests either that the population is young and will probably lose S-haplotypes in the future, or that low but recurrent levels of gene flow into the population are maintaining the observed haplotype diversity.

As we found 33 individuals with no S-haplotype identified in a total of 567 individuals (5.8%), we are certainly missing one or a few additional S-haplotypes. Because the number of gene copies observed for S-haplotypes ranged from 21 (AhS24) to 218 (AhS01), with a median around 100, it is likely that we did not miss
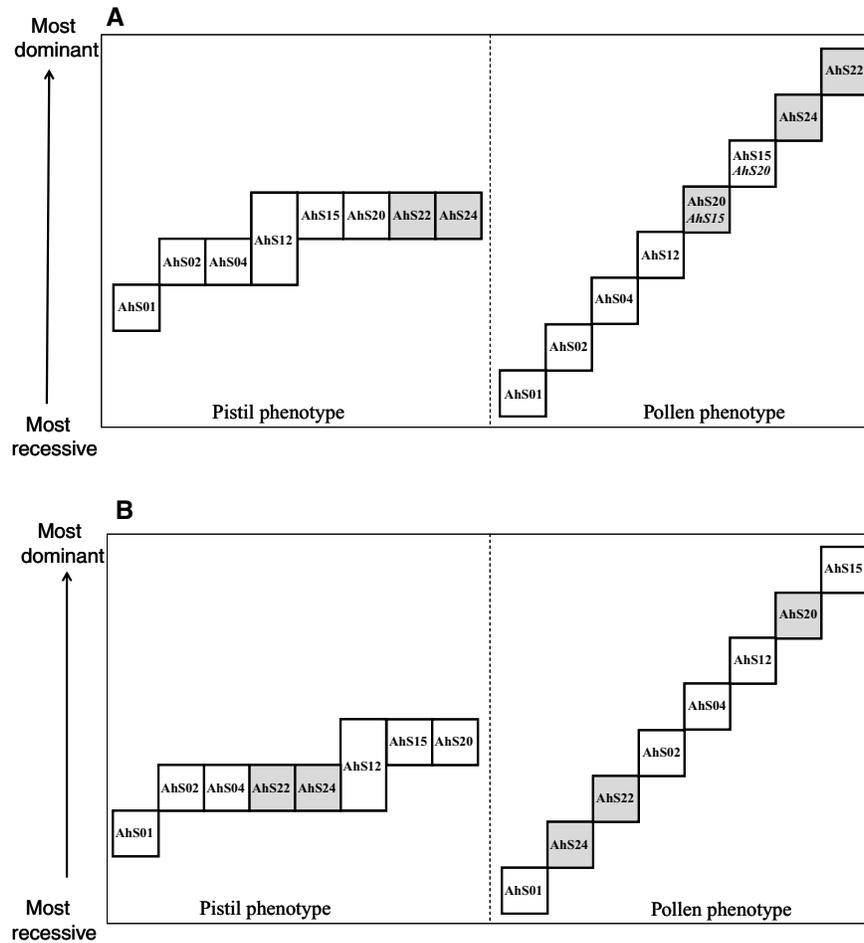
Figure 4. Putative alternative patterns of dominance interactions among S-haplotypes. S-haplotypes are represented by squares whose vertical position indicates relative dominance level. (A) Dominance interactions according to pattern 1 assuming three dominance classes in pistils and strictly hierarchical relationships in pollen with AhS22 as the most dominant and AhS24 as the second most dominant haplotype; alternative positions of AhS15 and AhS20 in the hierarchy in pollen according to pattern 2 are indicated in italics. (B) Dominance interactions according to pattern 3, which differs from pattern 1 with respect to the position of AhS22 and AhS24 at the bottom of the hierarchy in pollen and pistil.

more than two alleles, and hence the total number of S-haplotypes in this population should be around 10. Stochastic simulations showed that, assuming no immigration, the population should maintain fewer than eight haplotypes. This suggests that some level of migration would be needed to maintain the observed haplotype diversity. Indeed, using a paternity analysis, an upper estimate of pollen immigration rate of 10% was found in this population (Llaurens et al. 2008), which would be consistent with the observed number of S-haplotypes, taking into account that NFDS leads to high rates of effective migration (Schierup et al. 2000).

FREQUENCY CHANGE BETWEEN TWO SUCCESSIVE GENERATIONS

Table 5 shows the observed frequencies of S-haplotypes in the offspring population sample. The log-likelihoods under the four

Table 3. Likelihood of the fit of S-haplotype frequencies obtained by simulations under different selection models and patterns of dominance interactions (see text), to the observed data in *A. halleri*. Simulations parameters: population size $N=800$; mutation rate $\mu=10^{-6}$.

Selection model	Dominance interactions[1]	Log (likelihood)
Wright	pattern 1	−175.046
Wright	pattern 2	−176.853
Wright	pattern 3	−224.732
Wright	all codominant	−251.459
Fecundity selection	pattern 1	−189.067
Fecundity selection	pattern 2	−197.138
Fecundity selection	pattern 3	−228.572
Fecundity selection	all codominant	−251.541

[1] Described in Figure 4.

Table 4. Probability of loss of S-haplotypes in 10,000 simulation runs with population size $N=800$ and mutation rate $\mu=10^{-6}$ under Wright's and fecundity selection models with dominance interactions according to pattern 1 (see Fig. 4).

Selection model	Any haplotype	AhS01	AhS02	AhS04	AhS12	AhS15	AhS20	AhS22	AhS24
Fecundity selection	0.24	0	0	0	0	0	0	0.15	0.12
Wright	0.21	0.10	0.18	$1.\,10^{-3}$	$9.\,10^{-3}$	7.10^{-4}	0	0	0

models tested were respectively: (1) -31.41 for simulations with drift only (no SI system); (2) -38.6 with drift and NFDS on the S-locus with all S-haplotypes codominant; (3) -28.52 with drift and NFDS on the S-locus according to Wright's model and dominance interactions according to pattern 1; (4) -29.13 for the same case as (3) but under the fecundity selection model. Hence, implementing NFDS produced a better fit to the offspring data than a model with drift only, but the difference was significant only when comparing (1) and (3) ($\Delta AIC > 5$). Also, the assumption of strict codominance among S-haplotypes gave significantly lower fit to the offspring data than models allowing complex patterns of dominance interactions. The observed allele frequencies were consistent with the 95% boundaries obtained by simulations for all S-haplotypes except AhS04 (higher than expected), AhS22 (lower than expected), and AhS12 (slightly lower than expected). This suggests that additional processes not taken into account in the simulations may be influencing these S-haplotypes.

Discussion
ASSOCIATION BETWEEN *SRK* GENOTYPES AND SI PHENOTYPES
In an analysis of mating patterns within the same *A. halleri* population as the one studied here, Llaurens et al. (2008) reported 100% outcrossing, and a high number of successful paternal plants per

Table 5. Observed and expected frequencies of each S-haplotype in the offspring population sample. Simulations ($N=800$, $\mu=10^{-6}$) were performed under Wright's model of NFDS with dominance interactions according to dominance pattern 1 (see Fig. 4).

S-haplotype	Observed frequency	Expected 95% boundaries
AhS01	0.263	0.25–0.32
AhS02	0.065	0.05–0.09
AhS04	0.184[1]	0.08–0.13
AhS12	0.156[1]	0.16–0.22
AhS15	0.071	0.06–0.1
AhS20	0.148	0.14–0.2
AhS22	0.030[1]	0.04–0.08
AhS24	0.028	0–0.03

[1]Indicates that the observed frequency was outside the expected 95% boundaries obtained across 10,000 simulation replicates.

maternal individual. This suggests that SI is functional and that haplotype diversity at the S-locus is high enough to avoid constraints on mate availability. In total, 25 putative *SRK* alleles have been identified at the species level, but linkage to the S-locus and relationship to the incompatibility phenotype has only been demonstrated for a small fraction of these alleles. Our study is the first to use a comprehensive set of controlled pollinations to confirm the functionality of the SI system in *A. halleri* and to check exhaustively the consistency between *SRK* genotypes and the incompatibility phenotypes.

PATTERNS OF DOMINANCE INTERACTIONS AMONG S-HAPLOTYPES
Patterns of dominance interactions among S-haplotypes have been determined in pollen and pistil in surprisingly few species with sporophytic SI, and contrasting patterns were observed. In most species studied, codominant interactions were more frequent than dominant/recessive ones, and dominance occurred more frequently in pollen than in pistils (in Brassicaceae: *Brassica campestris*, Hatakeyama et al. 1998; *Brassica oleracea*, Ockendon 1975; *Raphanus raphanistrum*, Sampson 1967; *Sinapis arvensis*, Stevens and Kay 1989; or *Iberis amara*, Bateman 1954). These observations are in agreement with our results on *A. halleri*, except that we did not find evidence for codominant interactions in pollen phenotype. In contrast, identical patterns of dominance interactions in pollen and pistils were reported in other species (in Convolvulaceae *Ipomoea trifida*, Kowyama et al. 1994; and in Asteraceae *Senecio squalidus*, Brennan et al. 2002).

The patterns of dominance interactions determined in *A. halleri* are different from those in the sister species *A. lyrata* where identical dominance interactions in pollen and pistil phenotypes were reported, with dominance arranged hierarchically in four classes with several codominant haplotype per class (Prigoda et al. 2005). Several pairs of S-haplotypes from both species have very similar sequences and are probably derived from the same parental haplotype in their most recent common ancestor (V. Castric and X. Vekemans, unpubl. ms.). Such pairs of haplotypes probably retained SI, so one would expect the same dominance interactions in those trans-specific S-haplotypes. Five S-haplotypes found in our study are shared trans-specifically with *A. lyrata* haplotypes (V. Castric and X. Vekemans unpubl. ms.), that is, the *A. halleri*/*A. lyrata* pairs AhS01/AlS01; AhS02/AlS17; AhS04/AlS37, AhS12/AlS42, and AhS24/AlS20. The patterns of dominance

among these haplotypes in both species are strikingly similar for pistils, as expected. In pollen, however, we found a strict dominance hierarchy in *A. halleri* whereas in *A. lyrata,* codominant interactions were assumed between AlS17 and AlS37, as well as between the most dominant haplotypes AlS20 and AlS42, based on the relationship between phylogenetic clusters of SRK alleles and dominance levels (Prigoda et al. 2005). Although the overall dominance ranks of S-haplotypes are similar in both species in pollen, the absence of codominant interactions in *A. halleri* is puzzling. These differences could either be due to slight differences in the methodological procedures used to estimate dominance interactions, to variance in phenotype expression, or to evolutionary changes in patterns of dominance interactions between species. However, we observed high levels of concordance among replicates (most values in Fig. 1 are either close to 0 or 1); and the principal methodological difference, that is, the fact that we did not score as a separate category small fruits containing a few seeds and classified them as "incompatible," would lead to a slight overestimate of codominant interactions in pollen and is thus conservative with regards to codominance. However, before concluding that dominance interactions have evolved in either species, careful assessments for this and other pairs of trans-specific alleles in both species are needed.

Our results indicated strong correlation in the ranks of dominance levels in pollen and pistil phenotypes. This was also found in *A. lyrata* (Prigoda et al. 2005), but previous studies in the Brassicaceae often reported pairs of haplotypes with nonlinear (opposite) dominance interactions in pollen and pistils (Sampson 1967; Stevens and Kay 1989; Hatakeyama et al. 1998). Opposite dominance interactions in pollen and pistils would produce self-compatible genotypes in heterozygotes expressing one haplotype in pollen and the other in pistils. This suggests that this feature could perhaps explain the observed cases of "leaky" expression of SI, that is, presence of self-compatible plants, in those species (Stevens and Kay 1989; Nou et al. 1993). In our *A. halleri* population, the observation of strict outcrossing (Llaurens et al. 2008) tends to confirm the absence of such self-compatible genotype combinations.

NUMBER OF S-HAPLOTYPES
The level of haplotype diversity observed here is similar to those generally reported in species with sporophytic SI (six in *S. squalidus*, Brennan et al. 2002; 22 in a *R. raphanistrum*, Karron et al. 1990; 16 in *I. trifida*, Kowyama et al. 1994; 18 in *B. campestris*, Nou et al. 1993; three to 16 in different populations of *B. insularis*, Glémin et al. 2005; 12 in *A. lyrata*, Schierup et al. 2006) with the notable exception of a *S. arvensis* population which was found to contain 35 putative S-haplotypes (Stevens and Kay 1989). In comparison, within species with gametophytic SI,

a higher number of haplotypes are generally detected (Castric and Vekemans 2004). Theoretical studies of sporophytic SI showed that this pattern is expected as a consequence of the strong asymmetry in selection effects on recessive versus dominant alleles (Schierup et al. 1997; Billiard et al. 2007). Indeed, with $N = 800$, the number of S-haplotypes expected for a species with gametophytic SI is about 16 (computed according to Yokoyama and Hetherington 1982), which is about twice the value obtained in our simulations of sporophytic SI. The dominance interactions among S-haplotypes in sporophytic SI thus have a great influence on the S-locus haplotype diversity maintained in a population.

Our results also showed that the number of haplotypes in a dominance class correlates with dominance of that class in the pistil due to the lower equilibrium frequency and higher probability of invasion of dominant haplotypes, compared with recessive ones (Schierup et al. 1997). Similar observations have been reported in other species with sporophytic SI (*A. lyrata*, Schierup et al. 2006; *B. insularis*, Glémin et al. 2005).

EFFECT OF NFDS, PATTERNS OF DOMINANCE, AND GENETIC DRIFT ON S-HAPLOTYPE FREQUENCIES
Evidence for the effect of NFDS is demonstrated by the result that NFDS models provide a much better fit of the observed changes in haplotype frequencies between two generations than expectations based on genetic drift only. Of the two models of NFDS tested, we showed that S-haplotype frequencies in the two generations were closer to the predictions based on Wright's NFDS model than to those based on the fecundity selection model. The latter model assumes that NFDS is acting both on pistils and pollen, which could occur in ecological situations with pollen limitation, where fertilization success for pistils depends on the availability in compatible pollen donors (Vekemans et al. 1998). This agrees with results from Llaurens et al. (2008), which suggested that this population is well supplied with pollen, unlike fecundity selection model assumptions.

By explicitly taking genetic drift and patterns of dominance into account in simulations of sporophytic SI in a finite population, we showed that one S-haplotype (Ah24) lay outside the predicted range of frequencies in the parental population. The analysis of the change in frequency between two generations further pointed toward three S-haplotypes (AhS04, AhS12, and AhS22) whose frequencies changed more than expected in the finite population simulations. Several alternative explanations could explain these results. First, our knowledge of the number of S-haplotypes and their dominance interactions is not complete, and we were thus forced to make some assumptions for individuals with incomplete genotypes. Second, foundation or bottleneck events could have drastically modified haplotype frequencies in the population. This, however, cannot explain for the departure from expectation

in the offspring sample, which does not assume allele frequency equilibrium. Third, the hypothesis of selective equivalence of S-haplotypes in the NFDS model may be violated.

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