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# Unequal segregation of *SRK* alleles at the *S* locus in *Brassica cretica*

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## Summary

In the Brassicaceae plant family, which includes the *Arabidopsis* and *Brassica* genera, self-incompatibility (SI) is controlled by genes at the *S* locus. Using experimental crosses, we studied the pattern of inheritance of *S*-locus alleles in the wild species *Brassica cretica*. Four full-sib families were established and unequal segregation of alleles at the *SRK* SI gene was found in one family. The segregation distortion acted in favour of a recessive (class II) allele and was best explained by some form of gametic-level selection. Our findings are discussed in the light of theoretical predictions of differential accumulation of deleterious mutations among *S*-locus alleles.

## 1. Introduction

Self-incompatibility (SI) has evolved in many flowering plant families to avoid self-fertilization (de Nettancourt, 2001). SI is in most cases under genetic control by a single Mendelian locus, the *S* locus, which typically contains several genes (Silva & Goring, 2001). In the sporophytic type of SI (SSI), the SI phenotype of the haploid pollen is determined by the *S*-locus genotype of the diploid parent, the sporophyte (Richards, 1997). In the Brassicaceae family, which has the best-studied SSI system, two adjacent *S*-locus genes, *SRK* and *SCR*, determine the female (pistil) and male (pollen) SI specificities, respectively (Stein *et al*., 1991; Schopfer *et al*., 1999; Suzuki *et al*., 1999). The *SRK* gene is expressed as a trans-membrane receptor kinase in the stigma, where it recognizes the soluble gene product of its cognate *SCR*, which is expressed at the pollen surface (Takayama *et al*., 2001). The *SRK* and *SCR* genes are typically inherited as a single unit (Casselman *et al*., 2000), as recombination between the two determinant genes would disrupt the SI response (Uyenoyama & Newbigin, 2000). Indeed, population-based studies have found evidence for the near-absence of historical recombination between *SRK* and *SCR* (Kamau & Charlesworth, 2005; Charlesworth *et al*., 2006; Hagenblad *et al*., 2006; Takuno *et al*., 2007).

In SSI, there is typically a dominance relationship among *S*-locus alleles, which, for a given pair of alleles, may differ between the stigma and the pollen (Richards, 1997). In the *Brassica* genus, *S*-locus alleles belong to either of two dominance classes, where class I alleles are dominant over class II alleles in pollen but co-dominant in the stigma (Nasrallah *et al*., 1991). Dominance relationships among *S* alleles are more complex in *Arabidopsis* with four dominance classes being identified to date (Prigoda *et al*., 2005). SI is a classic example of a trait evolving under strong negative frequency-dependent selection with rare *S* alleles having more mating opportunities than more common alleles, because of the higher number of compatible mates available (Wright, 1939; Schierup, 1998). This situation accounts for the high levels of polymorphism typically observed at *S* loci (Lawrence, 2000). The relative fitness of a given *S* allele therefore depends mainly on its current frequency in the population. In SSI systems, however, the position of an allele in the dominance hierarchy also affects its fitness: a recessive *S* allele that is not expressed may 'escape' recognition in a particular mating and may thus attain a higher population frequency than a dominant allele – the so-called

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'recessive effect' (Sampson, 1974; Schierup *et al.*, 1997; Vekemans *et al.*, 1998; Uyenoyama, 2000; Billiard *et al.*, 2007).

Because of the special dynamics of *S* alleles in natural populations, the existence of additional fitness effects, over and above those determined by the frequency and dominance of *S* alleles, has been proposed. In particular, since frequency-dependent selection maintains *S* alleles in a population for periods of time much longer, on average, than alleles at neutral loci (Vekemans & Slatkin, 1994), *S* alleles may build up a significant genetic load by accumulating deleterious mutations (Uyenoyama, 2003). Also, due to the reduced rate of recombination in the *S*-locus region, each *S* allele will carry a unique array of mutations (Uyenoyama, 2003, 2005). Generally, purifying selection will eventually 'purge' deleterious mutations from the population, unless population size is very small (e.g. Gale, 1990). At the *S* locus, however, selective purging may be inefficient for two reasons: first, the typically high heterozygosity at the *S* locus will shelter recessive deleterious mutations from selection (Uyenoyama, 1997; Glémin *et al.*, 2001). Secondly, purifying selection against deleterious mutations, whether recessive or dominant, will be counteracted by the strong frequency-dependent selection favouring rare *S* alleles (Glémin *et al.*, 2001). As a consequence, the contribution from deleterious mutations to overall *S*-allele fitness may significantly influence equilibrium frequencies of *S* alleles in natural populations (Uyenoyama, 2003). It should be noted that the idea of an allele-specific mutational load applies more generally to loci in low-recombination regions experiencing balancing (including frequency-dependent) selection (Uyenoyama, 2005).

Because *S* alleles that carry a relatively large mutational load may suffer a segregation disadvantage compared with less affected *S* alleles, due to selection at the gametophytic (haploid) and/or zygotic stage, observations of unequal segregation ratios of *S* alleles in controlled crosses have been taken as evidence for the presence of deleterious mutations segregating at the *S* locus in natural populations of self-incompatible plants (Lawrence & Franklin-Tong, 1994; Bechsgaard *et al.*, 2004; Stone, 2004). In *Brassica*, indications of distorted segregation of markers linked to the *S* locus have been reported in conjunction with genetic map construction in cultivated *Brassica napus* (Cheung *et al.*, 1997) and *Brassica oleracea* (Camargo *et al.*, 1997), but no studies of *S*-locus segregation have been carried out in any wild *Brassica* species. Here, we investigate the segregation of alleles at the *SRK* gene in the wild species *Brassica cretica* using experimental crosses between parents with known genotypes.

Table 1. *Parental and offspring* SRK *genotypes in four full-sib* B. cretica *families. The parental genotypes are given as* $S_iS_j$ *(maternal parent)* × $S_kS_l$ *(paternal parent/pollen donor). Alleles* $S_{102}$–$S_{127}$ *and* $S_{202}$–$S_{206}$ *are class I and class II alleles, respectively*

| Family | Parental genotypes | Offspring genotypes | | | | Total |
|---|---|---|---|---|---|---|
| | | $S_iS_k$ | $S_iS_l$ | $S_jS_k$ | $S_jS_l$ | |
| 1 | $S_{120}S_{206} \times S_{102}S_{203}$ | 4 | 13 | 15 | 15 | 47 |
| 2 | $S_{124}S_{203} \times S_{120}S_{206}$ | 11 | 16 | 10 | 11 | 48 |
| 3 | $S_{124}S_{127} \times S_{127}S_{203}$ | 5 | 16 | 3 | 25 | 49 |
| 4 | $S_{124}S_{202} \times S_{112}S_{203}$ | 13 | 15 | 15 | 18 | 61 |

## 2. Materials and methods

### (i) *Plant material and crosses*

*B. cretica* is a diploid ($2n=18$) species occurring mainly in the Aegean region in the eastern Mediterranean (Snogerup *et al.*, 1990). It belongs, together with wild and cultivated *B. oleracea*, to a group of closely related *Brassica* species, which all carry the *Brassica* C-genome (Harberd, 1972; Kianian & Quiros, 1992; von Bothmer *et al.*, 1995). For the present study, four full-sib families were obtained from crosses made with plants originating from a *B. cretica* population at Moni Kapsa on eastern Crete (Table 1). The same plant was used as the maternal and paternal parent to families 1 and 2, respectively (Table 1), while the remaining six parents were unique. The parental plants were unrelated.

### (ii) *DNA extraction and* S-*locus genotyping*

Genomic DNA was extracted from young, fresh leaf tissue ground under liquid nitrogen and processed with the DNeasy Plant Mini kit (Qiagen) according to the instructions of the manufacturer. From each parental plant, approximately 1200 bp of the *SRK* kinase domain (exons 4–7) were amplified using primers specific for each of the two dominance classes. The primer pairs KD5, KD8 and KD4, KD7 were used to amplify class I and class II alleles, respectively. Separate amplification reactions were performed for each dominance class. For primer sequences see Park *et al.* (2002). The amplification reactions contained 1·5 mM $MgCl_2$, 0·25 mM dNTP, 1·5 $\mu$M of each primer and 0·3 U of AmpliTaq Gold DNA polymerase (Applied Biosystems) in a 20 $\mu$l reaction volume. The cycling scheme included 95 °C for 8 min, 30 cycles of 94 °C for 1 min, 56 °C for 2 min and 72 °C for 2 min, and finally 72 °C for 7 min. PCR products from parental individuals having both class I and class II alleles (I/II heterozygotes; six out of a total of seven distinct parents) were sequenced directly, whereas the PCR product from the single parent

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lacking class II alleles (apparent I/I homozygote) was first cloned in the pGEM-T vector system (Promega) before sequencing six positive clones. In both cases, sequencing was done in both directions using the original PCR primers and BigDye chemistry (Applied Biosystems). Sequences were assembled and edited in SeqMan (Lasergene6, DNAstar). Restriction enzyme recognition site differences between each pair of parents were identified from the *SRK* sequences obtained and offspring genotypes were determined by cleaving the *SRK* amplification product from each offspring individual with allele-specific restriction enzymes (*Bcl*I, *Ppi*I, *Dra*I and *Alu*I were used to distinguish class I alleles in families 1, 2, 3 and 4, respectively; *Hin*dIII were used for class II alleles). In this way, every allele segregating in each family could be unambiguously identified.

### (iii) *Data analysis*

Offspring *SRK* genotype data from each family were analysed within a maximum-likelihood framework following Bechsgaard *et al.* (2004). Briefly, the observed proportions of *SRK* genotypes among offspring in each of four families were fitted to three nested models describing alternative patterns of segregation of parental alleles among the offspring: (i) a Mendelian model with no free parameters where there are equal numbers of the four genotypes, (ii) a 'gametic' model with two free parameters where offspring genotype proportions are $(\frac{1}{2}+\alpha_1)(\frac{1}{2}+\alpha_2)$, $(\frac{1}{2}+\alpha_1)(\frac{1}{2}-\alpha_2)$, $(\frac{1}{2}-\alpha_1)(\frac{1}{2}+\alpha_2)$ and $(\frac{1}{2}-\alpha_1)(\frac{1}{2}-\alpha_2)$, where $\frac{1}{2}+\alpha_i$ and $\frac{1}{2}-\alpha_i$ denote the proportions in which the two alleles from parent $i$ segregate, and (iii) a 'zygotic' model with three free parameters where offspring genotype proportions are $x_1$, $x_2$, $x_3$ and $1-x_1-x_2-x_3$. The Mendelian model describes a situation with perfect Mendelian segregation. In the gametic model, alternate alleles from one or both of the parents are inherited in unequal proportions, presumably as a result of selection during the gametophytic (haploid) phase. In the zygotic model, which is fully parameterized, offspring proportions may take any value from 0 to 1; under this model, deviations from Mendelian proportions may be the result of selection during the gametophytic and/or sporophytic (zygotic) phase. For each model and family, parameter values were found that maximized the likelihood of the data. For each family, models were compared hierarchically by a likelihood-ratio test with $P$ values obtained from a $\chi^2$ distribution. The magnitude of segregation distortion was quantified by finding the maximum-likelihood value of $\alpha$ for each parent using a 'reduced' gametic model with one free parameter ($\alpha$); statistical significance was determined by comparing with a reduced Mendelian model in which the two alleles from a given parent segregated in equal proportions. For further details on model formulation and statistical procedures, see Bechsgaard *et al.* (2004).

Table 2. *Likelihood-ratio test of nested models of* SRK *allele segregation; $-2\Delta L$ denotes twice the difference in log-likelihood between the models compared. Boldface indicates significance at $P<0{\cdot}05$*

| | Gametic *vs.* Mendelian | | Zygotic *vs.* gametic | |
|---|---|---|---|---|
| Family | $-2\Delta L$ | $P$ | $-2\Delta L$ | $P$ |
| 1 | 5·377 | 0·068 | 3·283 | 0·070 |
| 2 | 1·504 | 0·471 | 0·227 | 0·634 |
| 3 | 25·317 | $\mathbf{3{\cdot}18\times10^{-6}}$ | 1·494 | 0·222 |
| 4 | 0·821 | 0·663 | 0·006 | 0·939 |

## 3. Results

Between 47 and 61 offspring individuals from the four families were successfully genotyped (Table 1). In families 1, 2 and 4, both parents carried one *SRK* class I allele and one *SRK* class II allele (I/II heterozygotes; Table 1). In family 3, the maternal parent carried two *SRK* class I alleles (I/I homozygote), whereas the paternal parent was a I/II heterozygote (Table 1). The two class I alleles of the maternal parent in family 3 were, however, different; thus all parental individuals were *S*-locus heterozygotes. There was a total of five distinct class I alleles, and three distinct class II alleles, among the seven different parents (Table 1).

In two of the families (families 2 and 4), *S*-allele segregation conformed to a Mendelian model, whereas family 3 displayed a significantly skewed segregation pattern (Table 2). The deviation from Mendelian proportions in family 3 was not better explained by a parameter-rich zygotic model than by a reduced gametic model, as determined from the non-significant likelihood difference between the two models (Table 2). In the remaining family (family 1), the deviation from a Mendelian segregation ratio was close to being significant at the 5% level (Table 2). This family also showed the highest likelihood difference between the zygotic and gametic models, but, again, the difference failed to reach significance (Table 2). Analysing the segregation of *SRK* alleles from each parent separately, we found a significant segregation advantage of the class II allele $S_{203}$ over the class I allele $S_{127}$ in the paternal parent of family 3 ($P=8{\cdot}2\times10^{-7}$, $\alpha=-0{\cdot}337$; Table 3), and a nearly significant segregation advantage of the class II allele $S_{206}$ over the class I allele $S_{120}$ in the maternal parent of family 1 ($P=0{\cdot}0563$, $\alpha=-0{\cdot}138$; Table 3). No significant segregation distortion was detected in any of the remaining parents, but we note that in all but

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Table 3. *Likelihood-ratio test of nested models of* SRK *allele segregation in individual parents; $-2\Delta L$ denotes twice the difference in log-likelihood between the models compared;* α *is the magnitude of segregation distortion, the value of which refers to the first allele in each comparison. Boldface indicates significance at* $P<0{\cdot}05$

| | | Gametic *vs.* Mendelian | | | |
|---|---|---|---|---|---|
| Family | Alleles | $-2\Delta L$ | $P$ | $\alpha$ | Favoured allele |
| 1 | $S_{120}$ *vs.* $S_{206}$ | 3·643 | 0·0563 | −0·138 | $S_{206}$ |
| | $S_{102}$ *vs.* $S_{203}$ | 1·734 | 0·1879 | −0·096 | $S_{203}$ |
| 2 | $S_{124}$ *vs.* $S_{203}$ | 0·752 | 0·3859 | 0·063 | $S_{124}$ |
| | $S_{120}$ *vs.* $S_{206}$ | 0·752 | 0·3859 | −0·063 | $S_{206}$ |
| 3 | $S_{124}$ *vs.* $S_{127}$ | 1·003 | 0·3165 | −0·071 | $S_{127}$ |
| | $S_{127}$ *vs.* $S_{203}$ | 24·314 | $\mathbf{8{\cdot}2\times10^{-7}}$ | −0·337 | $S_{203}$ |
| 4 | $S_{124}$ *vs.* $S_{202}$ | 0·410 | 0·5218 | −0·041 | $S_{202}$ |
| | $S_{112}$ *vs.* $S_{203}$ | 0·410 | 0·5218 | −0·041 | $S_{203}$ |

one of the class I/II heterozygous parents, segregation was in favour of the class II allele (Table 3).

## 4. Discussion

We found a large and significant deviation from strict Mendelian segregation at the *S* locus in family 3 and a strong tendency towards non-Mendelian *S*-locus segregation in family 1. In both families, but less obviously so in family 1, the deviation could best be explained by a gametic model, indicating that selection has acted during the haploid gametic stage (Table 2). Gametic selection acting on *S* alleles has been inferred as the major cause of *S*-locus segregation distortion also in *Papaver rhoeas* (Lawrence & Franklin-Tong, 1994) and in *Arabidopsis lyrata* (Bechsgaard *et al.*, 2004). These findings suggest that if *S*-locus segregation distortion is caused by accumulated deleterious mutations, as theory predicts, then these mutations are most likely expressed during the gametophytic stage. Even though a skewed segregation ratio is often observed at marker loci dispersed throughout the genome (e.g. in genetic mapping studies), segregation distortion at the *S* locus appears to be particularly pronounced. In a recent study, Leppälä *et al.* (2008) found a higher rate of distortion at the *S* locus compared with the average of 16 unlinked markers distributed across all eight chromosomes in *A. lyrata*. In concordance with theory (e.g. Uyenoyama, 2003), this finding implies that the particular evolutionary dynamics of the *S* locus (negative frequency-dependent selection and a low regional rate of recombination) will lead to an allele-specific mutational load.

Only the alleles from the paternal parent segregated unequally in family 3 (Table 3). Among these alleles, the $S_{203}$ allele had a segregation advantage over the $S_{127}$ allele (Table 3). $S_{203}$ is a class II recessive allele, while the alternative paternal allele is a class I dominant allele. Indeed, in six of the seven parents that were class I/II heterozygotes, the recessive class II allele had a segregation advantage (even if this was significant in one parent only; Table 3). Similarly, Bechsgaard *et al.* (2004) found that segregation advantage accrued mainly to recessive *S* alleles in *A. lyrata*. In SSI species such as *B. cretica* and *A. lyrata*, dominant *S* alleles cannot appear in homozygous form, whereas recessive alleles can. For this reason, recessive *S* alleles can be expected to carry fewer recessive deleterious mutations since such mutations are more likely to be purged by selection when homozygous (Uyenoyama, 1997; Glémin *et al.*, 2001). Moreover, dominant *S* alleles are expected to accumulate more dominant deleterious mutations by random drift due to their smaller effective population sizes compared with recessive *S* alleles (Bechsgaard *et al.*, 2004). The combined effect would be a higher overall mutational load for dominant *S* alleles, which would explain the observations of unequal segregation in favour of recessive *S* alleles observed in both *B. cretica* and *A. lyrata*.

We found that the *B. cretica* $S_{203}$ allele apparently enjoys a substantial segregation advantage, at least in one of the four parents in which it occurred (Table 3). Segregation advantages of similar magnitude have been reported from *A. lyrata* (Bechsgaard *et al.*, 2004). These results clearly indicate that the additional selective advantage provided by segregation distortion may significantly influence the frequencies of *S* alleles in natural populations. In *A. lyrata*, alleles favoured by segregation have been reported to be present in higher than expected number in a natural population (Bechsgaard *et al.*, 2004; Schierup *et al.*, 2006). In *B. cretica*, ongoing investigations indicate that the $S_{203}$ and $S_{206}$ alleles are indeed among the most common in natural populations of this species (K. Edh, unpublished results).

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Nevertheless, care should be taken when extrapolating results from a limited set of experimental crosses to the situation in natural populations, as the segregation advantage of a given *S* allele may differ among families. The *B. cretica* $S_{203}$ allele, for example, is present in all four families, but showed a significant segregation advantage in one family only (Table 3). The same phenomenon has also been observed in *A. lyrata* (Bechsgaard *et al.*, 2004). Presumably, the absolute magnitude of segregation distortion in a certain family depends on the difference in mutational load between the two alleles in each parent.

Finally, we note that in family 3, both parents possessed a copy of the class I *SRK* allele $S_{127}$ (Table 1). Given that class I alleles are pollen dominant and stigma co-dominant, this cross should have been incompatible. We see a few possible explanations why this was not the case. First, even though the maternal and paternal $S_{127}$ alleles were identical with respect to the sequenced part of the kinase domain of the *SRK* gene, the alleles might differ in the *S*-domain, which codes for the part of the SRK protein directly involved in recognition (Kachroo *et al.*, 2001). We do not find this possibility very likely, however, since data from cultivated *Brassica* species show that *SRK* alleles carrying different SI specificities with a few exceptions differ substantially also at the kinase domain of *SRK* (Awadalla & Charlesworth, 1999; Takebayashi *et al.*, 2003). Secondly, the class I $S_{127}$ allele could be recessive to the class II $S_{203}$ in the pollen parent. However, class I alleles are always dominant over class II alleles in the close relative *B. oleracea*, and this dominance relationship appears to be the rule also in wild *Brassica* species (Glémin *et al.*, 2005; K. Edh, unpublished results). Thirdly, $S_{127}$ may be a non-functional allele. We find this hypothesis the most likely for the following reasons: The *SRK* nucleotide sequence at exons 4–7 of the *B. cretica* $S_{127}$ allele is very similar to the *B. oleracea* *S*-28 allele (K. Edh, unpublished results), which, in turn, is similar to the dominant class I *S* allele *S-f*2 in *Brassica rapa* (Fujimoto *et al.*, 2006), indicating that *B. rapa S-f*2, *B. oleracea S*-28 and *B. cretica* $S_{127}$ belong to the same allelic lineage (and share the same basic recognition specificity). Furthermore, Fujimoto *et al.* (2006) observed that in *B. rapa* individuals heterozygous for *S-f*2 and the recessive class II $S_{60}$, allele transcription of both alleles was abolished leading to complete absence of *S*-genotype expression and concomitant loss of SI. Thus, even though the evidence is clearly circumstantial, the *B. cretica* $S_{127}$ allele and the *B. rapa S-f*2 allele may carry the same structural mutations that make them non-functional, explaining why the cross producing family 3 was compatible. Indeed, upon enforced self-pollination, all the offspring in family 3 produced seed (A. Ceplitis, unpublished results). However, whether the $S_{127}$ itself is non-functional, or linked to a different locus conferring self-compatibility, cannot be determined from the family 3 data alone. In fact, the *B. oleracea S*-28 is a fully functional allele. Since *B. cretica* is more closely related to *B. oleracea* than to *B. rapa*, shared mutations between *B. cretica* and *B. rapa S* alleles would be unexpected, but might have been caused by incomplete lineage sorting during speciation. This hypothesis must, however, be verified by more detailed studies of *SRK* expression in *B. cretica*. Nevertheless, even if the $S_{127}$ allele were non-functional, we should expect equal genotypic proportions in the offspring in the absence of segregation distortion. Hence, whatever the reason for the compatibility between the parents of family 3, it does not alter the conclusion of a significant segregation distortion of *SRK* alleles in this family. Together with previous studies, our results suggest that segregation distortion at the *S* locus might be a widespread phenomenon with a potentially significant influence on *S*-allele frequencies in natural populations.

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## References

Awadalla, P. & Charlesworth, D. (1999). Recombination and selection at *Brassica* self-incompatibility loci. *Genetics* **152**, 413–425.

Bechsgaard, J., Bataillon, T. & Schierup, M. H. (2004). Uneven segregation of sporophytic self-incompatibility alleles in *Arabidopsis lyrata*. *Journal of Evolutionary Biology* **17**, 554–561.

Billiard, S., Castric, V. & Vekemans, X. (2007). A general model to explore complex dominance patterns in plant sporophytic self-incompatibility systems. *Genetics* **175**, 1351–1369.

Camargo, L. E. A., Savides, L., Jung, G., Nienhuis, J. & Osborn, T. C. (1997). Location of the self-incompatibility locus in an RFLP and RAPD map of *Brassica oleracea*. *Journal of Heredity* **88**, 57–60.

Casselman, A. L., Vrebalov, J., Conner, J. A., Singhal, A., Giovannoni, J., Nasrallah, M. E. & Nasrallah, J. B. (2000). Determining the physical limits of the *Brassica S* locus by recombinational analysis. *Plant Cell* **12**, 23–33.

Charlesworth, D., Kamau, E., Hagenblad, J. & Tang, C. L. (2006). *Trans*-specificity at loci near the self-incompatibility loci in *Arabidopsis*. *Genetics* **172**, 2699–2704.

Cheung, W. Y., Champagne, G., Hubert, N., Tulsieram, L., Charne, D., Patel, J. & Landry, B. S. (1997). Conservation of *S*-locus for self-incompatibility in *Brassica napus* (L.) and *Brassica oleracea* (L.). *Theoretical and Applied Genetics* **95**, 73–82.

de Nettancourt, D. (2001). *Incompatibility and Incongruity in Wild and Cultivated Plants*. Berlin: Springer.

Fujimoto, R., Sugimura, T., Fukai, E. & Nishio, T. (2006). Suppression of gene expression of a recessive *SP11/SCR* allele by an untranscribed *SP11/SCR* allele in *Brassica* self-incompatibility. *Plant Molecular Biology* **61**, 577–587.

https://doi.org/10.1017/S001667230800935X Published online by Cambridge University Press

Gale, J. S. (1990). *Theoretical Population Genetics*. London: Unwin Hyman.
Glémin, S., Bataillon, T., Ronfort, J., Mignot, A. & Olivieri, I. (2001). Inbreeding depression in small populations of self-incompatible plants. *Genetics* **159**, 1217–1229.
Glémin, S., Gaude, T., Guillemin, M.-L., Lourmas, M., Olivieri, I. & Mignot, A. (2005). Balancing selection in the wild: testing population genetics theory of self-incompatibility in the rare species *Brassica insularis*. *Genetics* **171**, 279–289.
Hagenblad, J., Bechsgaard, J. & Charlesworth, D. (2006). Linkage disequilibrium between incompatibility locus region genes in the plant *Arabidopsis lyrata*. *Genetics* **173**, 1057–1073.
Harberd, D. J. (1972). A contribution to the cyto-taxonomy of *Brassica* (Cruciferae) and its allies. *Botanical Journal of the Linnean Society* **65**, 1–23.
Kachroo, A., Schopfer, C. R., Nasrallah, M. E. & Nasrallah, J. B. (2001). Allele-specific receptor–ligand interactions in *Brassica* self-incompatibility. *Science* **293**, 1824–1826.
Kamau, E. & Charlesworth, D. (2005). Balancing selection and low recombination affect diversity near the self-incompatibility loci of the plant *Arabidopsis lyrata*. *Current Biology* **15**, 1773–1778.
Kianian, S. F. & Quiros, C. F. (1992). Trait inheritance, fertility, and genomic relationships of some $n=9$ *Brassica* species. *Genetic Resources and Crop Evolution* **39**, 165–175.
Lawrence, M. J. (2000). Population genetics of the homomorphic self-incompatibility polymorphisms in flowering plants. *Annals of Botany* **85**, 221–226.
Lawrence, M. J. & Franklin-Tong, V. E. (1994). The population genetics of the self-incompatibility polymorphism in *Papaver rhoeas*. 9. Evidence of an extra effect of selection acting on the *S*-locus. *Heredity* **72**, 353–364.
Leppälä, J., Bechsgaards, J. S., Schierup, M. H. & Savolainen, O. (2008). Transmission ratio distortion in *Arabidopsis lyrata*: effects of population divergence and the *S*-locus. *Heredity* **100**, 71–78.
Nasrallah, J. B., Nishio, T. & Nasrallah, M. E. (1991). The self-incompatibility genes of *Brassica*: expression and use in genetic ablation of floral tissues. *Annual Review of Plant Physiology and Plant Molecular Biology* **42**, 393–422.
Park, J. I., Lee, S. S., Watanabe, M., Takahata, Y. & Nou, I. S. (2002). Identification of *S*-alleles using polymerase chain reaction-cleaved amplified polymorphic sequence of the *S*-locus receptor kinase in inbreeding lines of *Brassica oleracea*. *Plant Breeding* **121**, 192–197.
Prigoda, N. L., Nassuth, A. & Mable, B. K. (2005). Phenotypic and genotypic expression of self-incompatibility alleles in *Arabidopsis lyrata* suggests unique origin of alleles in different dominance classes. *Molecular Biology and Evolution* **22**, 1609–1620.
Richards, A. J. (1997). *Plant Breeding Systems*. London: Chapman and Hall.
Sampson, D. R. (1974). Equilibrium frequencies of sporophytic self-incompatibility alleles. *Canadian Journal of Genetics and Cytology* **16**, 611–618.
Schierup, M. H. (1998). The number of self-incompatibility alleles in a finite, subdivided population. *Genetics* **149**, 1153–1162.
Schierup, M. H., Vekemans, X. & Christiansen, F. B. (1997). Evolutionary dynamics of sporophytic self-incompatibility alleles in plants. *Genetics* **147**, 835–846.
Schierup, M. H., Bechsgaard, J. S., Nielsen, L. H. & Christiansen, F. B. (2006). Selection at work in self-incompatible *Arabidopsis lyrata*: mating patterns in a natural population. *Genetics* **172**, 477–484.
Schopfer, C. R., Nasrallah, M. E. & Nasrallah, J. B. (1999). The male determinant of self-incompatibility in *Brassica*. *Science* **286**, 1697–1700.
Silva, N. F. & Goring, D. R. (2001). Mechanisms of self-incompatibility in flowering plants. *Cellular and Molecular Life Sciences* **58**, 1988–2007.
Snogerup, S., Gustavsson, M. & von Bothmer, R. (1990). *Brassica* sect. *Brassica* (Brassicaceae). I. Taxonomy and variation. *Willdenowia* **19**, 271–365.
Stein, J. C., Howlett, B., Boyes, D., Nasrallah, M. E. & Nasrallah, J. B. (1991). Molecular cloning of a putative receptor protein kinase gene encoded at the self-incompatibility locus of *Brassica oleracea*. *Proceeding of the National Academy of Sciences of the USA* **88**, 8816–8820.
Stone, J. L. (2004). Sheltered load associated with *S*-alleles in *Solanum carolinense*. *Heredity* **92**, 335–342.
Suzuki, G., Kai, N., Hirose, T., Fukui, K., Nishio, T., Takayama, S., Isogai, A., Watanabe, M. & Hinata, K. (1999). Genomic organisation of the *S* locus: identification and characterization of genes in SLG/SRK region of S-9 haplotype of *Brassica campestris* (syn. *rapa*). *Genetics* **153**, 391–400.
Takayama, S., Shimosato, H., Shiba, H., Funato, M., Che, F.-S., Watanabe, M., Iwano, M. & Isogai, A. (2001). Direct ligand–receptor complex interactions controls *Brassica* self-incompatibility. *Nature* **413**, 534–538.
Takebayashi, N., Brewer, P. B., Newbigin, E. & Uyenoyama, M. K. (2003). Patterns of variation within self-incompatibility loci. *Molecular Biology and Evolution* **20**, 1778–1794.
Takuno, S., Fujimoto, R., Sugimura, T., Sato, K., Okamoto, S., Zhang, S. L. & Nishio, T. (2007). Effects of recombination and hitchhiking diversity in the *Brassica* self-incompatibility locus complex. *Genetics* **177**, 949–958.
Uyenoyama, M. K. (1997). Genealogical structure among alleles regulating self-incompatibility in natural populations of flowering plants. *Genetics* **147**, 1389–1400.
Uyenoyama, M. K. (2000). Evolutionary dynamics of self-incompatibility alleles in *Brassica*. *Genetics* **156**, 351–359.
Uyenoyama, M. K. (2003). Genealogy-dependent variation in viability among self-incompatibility genotypes. *Theoretical Population Biology* **63**, 281–293.
Uyenoyama, M. K. (2005). Evolution under tight linkage to mating type. *New Phytologist* **165**, 63–70.
Uyenoyama, M. K. & Newbigin, E. (2000). Evolutionary dynamics of dual-specificity self-incompatibility alleles. *Plant Cell* **12**, 310–311.
Vekemans, X. & Slatkin, M. (1994). Gene and allelic genealogies at a gametophytic self-incompatibility locus. *Genetics* **137**, 1157–1165.
Vekemans, X., Schierup, M. H. & Christiansen, F. B. (1998). Mate availability and fecundity selection in multi-allelic self-incompatibility systems in plants. *Evolution* **52**, 19–29.
von Bothmer, R., Gustavsson, M. & Snogerup, S. (1995). *Brassica* sect. *Brassica* (Brassicaceae). II. Inter- and intraspecific crosses with cultivars of *B. oleracea*. *Genetic Resources and Crop Evolution* **42**, 165–178.
Wright, S. (1939). The distribution of self-sterility alleles in populations. *Genetics* **24**, 538–552.

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