How can natural hybridisation between self-compatible and self-incompatible species be bi-directional?

Chengjun Li, Roger D Cousens*, Mohsen B Mesgaran†

School of Biosciences, The University of Melbourne, 3010, Melbourne, Victoria, Australia.

† Current address: Department of Plant Sciences, University of California, Davis, 95616, California, USA.

* Author for correspondence. Email: rcousens@unimelb.edu.au.

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Summary

Self-compatible (SC) plants often deposit pollen on their own stigmas before flowers open. Thus, if we find natural hybrids between a SC species and a closely-related self-incompatible (SI) species, the pollen donor is usually expected to be the SC species. However, in previous studies of naturally-occurring mixed populations of invasive *Cakile maritima* (SI) and *Cakile edentula* (SC) in Australia, hybridisation was found to have been bi-directional. Here, we explored the floral development of these species in order to test the hypothesis that there might be a short opportunity for pollinators to visit open SC flowers before the self-pollen is released. Results indicated that both species showed reverse herkogamy during early development, though by the time pollen was ripe the four long anthers always over-topped the stigma; in *C. maritima* most of the short anthers remained below the stigma. In both species, ripe, compatible pollen was able to germinate on the stigmatic surface at all stages of floral development. Anther dehiscence commenced earlier in *C. edentula* and well before flowers opened, in contrast to *C. maritima*, but the timing of pollen release varied between individual flowers, with a small proportion of uncontaminated stigmas after flowering; therefore, *C. edentula* can be either pollen recipient or pollen donor when inter-specific outcrossing occurs. In summary, our results suggest that the variation between individual flowers of *C. edentula* contributed to the bi-directional hybridisation despite being a predominant selfer, providing new insights into plant colonisation.

Keywords: *Cakile*, bi-directional hybridisation, self-(in) compatibility, stigma receptivity, anther dehiscence
Introduction

Hybrids may occur naturally where the habitats of closely related species are separated by short distances or where related species from geographically distinct regions are introduced into the same habitat. The occurrence of hybrid genotypes may have consequences for local adaptation, speciation, colonisation, range shifts and species displacement (Yakimowski & Rieseberg, 2014) but this varies considerably. At one extreme, hybrids may have no impact whatsoever on the long-term success of the species, while at the other extreme hybrid demography and adaptation could be essential for one or both parental species. The outcome of hybridisation depends on a range of factors, including their relative reproductive outputs, attractiveness to pollinators, phenology, abundance, mating system, and the viability, vigour and fertility of hybrid offspring (Goodwillie & Ness, 2013).

However, the role of mating systems in the dynamics of hybridising species has seldom been considered (Bouhours et al., 2017); if wild hybrids are detected, either morphologically or genetically (Ohadi et al., 2015), there must have been at least some level of compatibility between the species’ mating systems. Mating systems, however, are frequently considered to be crucial for colonisation by a single species (Pannell, 2015). Traits promoting self-fertilisation — temporal, physiological, mechanical or a combination of these (Barrett & Harder, 2017) — are likely to be a demographic advantage if just one or a few offspring arrive at a distant location (Cheptou, 2012). The reproductive assurance provided by the ability to self-fertilise is thus argued to be the reason that greater relative frequencies of autogamous species are found on islands compared to large land masses and in invasive species compared with native floras (Petanidou et al., 2012). Notwithstanding the almost universal acceptance of “Baker’s rule”, i.e., plant species with self-compatibility are more likely to become successfully colonists than species that are self-incompatible (Pannell et al., 2015), there are a great many successful, non-selfing invaders (Ridley & Ellstrand, 2009).

The role of plant mating systems to hybridisation can be complex. When two species share a pollen vector, a stigma can potentially receive pollen from other plants of either species, from any existing hybrids and from its own flowers. In animal-pollinated species, actual deposition of pollen types — both quantity and relative frequency — will depend on the floral preferences of the pollinators at the time and the abundance of the different pollen donors. Pollinators tend to be more attracted by the larger and more rewarding flowers of out-crossing species compared with those in which selfing predominates (Willmer, 2011). There may be temporal and structural differences between species in floral development that affect the
34 proportions and relative timing of self- and non-self pollen deposition. Flowers may only open, if at all, after their own pollen has begun being released, ensuring preferential deposition of self-pollen. Which pollen grain successfully fertilises an ovule, i.e. the outcome of “pollen competition”, will depend on its relative time of arrival, the developmental stage of the stigma, the rate of its pollen tube growth and the innate physiological compatibility between the pollen and the stigma — all of which may interact. Whether the resulting zygote becomes a viable, reproductive plant will also depend on a series of other, post-zygotic steps (Hao et al., 2012).

42 In addition to mating system determining whether two species can form hybrids, it may also determine the direction in which hybridisation can occur. Unilateral incompatibility is the phenomenon where the pollen of one species is rejected by the stigma of the other, whereas the reciprocal cross is successful (Hiscock & Dickinson, 1993). This has been reported in hand-crosses within multiple families, usually where the successful pollen donor species is self-compatible (SC) and the recipient is physiologically self-incompatible (SI); it is also referred to as the SI x SC rule (Lewis & Crowe, 1958). The mechanism of self-incompatibility has been implicated in unilateral (interspecific) incompatibility with other members of the Brassicaceae (Kitashiba & Nasrallah, 2014).

51 We have been studying self-compatible Cakile edentula (Bigelow) Hook. and self-incompatible Cakile maritima Scop (Thrall et al., 2000), closely related Brassicaceae occupying the same narrow coastal habitat (Cousens et al., 2013). C. edentula arrived in Australia first and spread widely but has now been replaced over much of its range by C. maritima (Rodman, 1986); similar arrival and replacement occurred in California (Boyd & Barbour, 1993) and on the North Island of New Zealand (Cousens et al., 2013). Putative hybrid phenotypes have been found in the current sympatric zone in Tasmania (Cousens & Cousens, 2011). Unilateral incompatibility is quite common in the Brassicaceae (Hiscock & Dickinson, 1993), although most of the examples are from inter-generic crosses. However, it does not seem to apply to these two Cakile species. Hand-crosses of emasculated flowers are usually successful in both directions in the glasshouse (Mesgaran et al., 2016). Chloroplast markers also showed that introgression previously occurred from C. edentula to C. maritima, and vice versa (Ohadi et al., 2015). This field evidence is surprising in light of a report that C. edentula is cleistogamous (Meehan, 1892). The report might be not so plausible since many flowers in C. edentula remain open despite some of them being enclosed without petals (pers. obs.); nevertheless, a large proportion of flowers have been contaminated by self-pollen even before flowering (see Results section). Moreover, selfing rate in C. edentula is relatively high (Donohue, 1998), with
over 80% of flowers setting fruits either in the field or under glasshouse conditions (unpubl. data). Therefore, if its stigma has already been contaminated by its pollen before alien pollen arrives, how can *C. edentula* become the maternal parent of hybrids?

There are several possible explanations. It may be that although *C. edentula* pollen is deposited within buds, the stigma may not be receptive until the flower fully opens; if in-bud pollen release only results from agitation by wind, perhaps the pollen itself may not be viable at that time. In these cases, there may still actually be a narrow temporal “window of opportunity” between the *C. edentula* flower opening and its own pollen fertilising, in which an insect could deposit non-self pollen. *C. edentula* flowers do not remain closed and insects have been observed going from one *Cakile* species to the other in mixed natural and artificial populations (Mesgaran et al., 2016) — although visitation is much less frequent in *C. edentula* than *C. maritima*. It is also possible that herkogamy — separation of stigmas and stamens spatially— could occur in *C. edentula* such that self-pollen cannot be transferred passively to the stigma surface without the assistance of pollinators at some point (Glover, 2014).

In this study, we explored the temporal (i.e. synchrony between the male and female functions) and spatial (herkogamy) separation of male and female functions in flowers of these *Cakile* species. The timing of pollen ripening, pollen dehiscence, stigma receptivity and structural positions of anthers and stigma in Australian populations of these two *Cakile* species were measured. By doing so, we addressed the following questions: (1) is there any window (temporal or spatial) of opportunity for pollinators to facilitate hybridisation; and if not, what is the nature of the window of opportunity? (2) How does such a window of opportunity contribute to the bi-directional hybridisation between species and what are the implications for plant colonisation?

**Materials and Methods**

Seeds were collected from Ulverstone (*C. maritima*) and Sloping Main (*C. edentula*) in Tasmania, Australia in 2013, north and south of the sympatric region respectively (Ulverstone is at least 500 km along the coast from the sympatric zone and no plants exhibited morphological characters of *C. edentula*). Seeds of both species from the sympatric zone (Raspins Beach) were also collected to include inter-population variation. Seeds were placed on moistened filter paper in Petri dishes and incubated in a cold room at 5 °C for 7-10 days. Pre-treated seeds were sown in pots in a polythene tunnel at Burnley Campus, the University of Melbourne. Seeds of each species from each population were germinated and evenly sown into 10 pots; seedlings were thinned to one per pot after four weeks, adding up to 40 plants in
Bud development

Bud development was assessed and defined into different stages as referred to Smyth et al. (1990). Buds were sampled sequentially along a random raceme on each plant, reflecting the temporal sequence of their initiation, to assess the bud development of each species. They were dissected and the lengths of the pistil and stamens were measured using ImageJ (https://imagej.nih.gov/ij/) with photographs taken under a dissecting microscope (Leica M205A, Leica Microsystems Pty Ltd). Landmark events were then identified and used to categorise sampled buds into corresponding stages. This was repeated on three racemes for each plant. Only one bud was sampled for each developmental stage on each raceme, adding up to 60 buds per stage per species (1 bud × 3 racemes × 20 plants) and 960 buds in total. When collecting data, extremely small buds were discarded due to the failure of dissecting them under the microscope. Fully-opened flowers were also not considered since it was obvious that the stigma surface in both species was heavily covered with pollen during this period. Data were analysed using SAS (Version 9.4; SAS Institute, Cary, NC) with PROC MIXED with “stage” as the fixed variable and “plant” and “population” as the random variables.

Anther dehiscence

Several commonly used solid pollen growth media (SPGMs) for Arabidopsis pollen were tested with the two Cakile species but were found to be ineffective in determining pollen ripeness (see details in Boavida & McCormick, 2007). Instead, rupture of the anther endothecium, a pre-condition for pollen dehiscence, was recorded under a dissecting microscope. Anthers of buds dissected previously were used to estimate the degree of anther dehiscence.

Stigmatic receptivity

Stigma receptivity of each species was recorded as the occurrence of pollen tube growth following artificial application of ripe pollen. After dissection and assessing their corresponding developmental stages, the same samples (i.e., three buds per stage per plant) used in bud development were emasculated and immediately inserted into an agar pad, standing upright with the pedicel in the agar (see details in Edlund et al., 2016). The agar pad was made from 1.5% agarose in distilled water. A newly-open flower from another plant of the same species was used as the source of pollen, minimising the possible effects of self-incompatibility. A freshly dehisced anther was removed and brushed across the target stigma, spreading pollen.
evenly. Two hours later, the pistil was removed, placed in a 1.5 mL tube containing 150 μl of a fixing solution (9:1 distilled water:glacial acetic acid), and stored at 4 °C. When required, the fixing solution was replaced with 1 M NaOH and heated at 50 °C for 1 h to soften the tissue and to make the pollen tubes more accessible to the dye. The pistil was washed gently three times with 50 mM KPO$_4$ buffer and then stained in 0.01% aniline ABF (Aniline Blue Fluorescence: Biosupplies, http://www.biosupplies.com.au) for 5-10 min. It was then placed in a drop of 50% glycerol on a microscope slide, covered with a cover slip and squashed slightly so that it became flat. Pollen tubes were photographed using a fluorescence microscope (Leica DM 2500, Leica Microsystems Pty Ltd) under UV light (wavelength = 495 nm). If fewer than five pollen tubes were observed, the stigma was assumed to be not yet receptive or only just becoming so. Otherwise, numbers of pollen tubes from 5-10, 10-20 and above 20 were classified as low (1), medium (2) and high (3), respectively. A Generalized Linear Mixed Model (GLMM) (PROC GLIMMIX in SAS 9.4; SAS Institute, Cary, NC) was used to analyse the data with degree of pollen tube growth as the response variable, assuming a multinomial distribution and using cumulative logit as the link function. “stage” was the fixed variable while “plant” and “population” were treated as random variables. Odds ratios were compared between stages.

Results

Bud development

Bud development in both species can be categorised into a simple descriptive 8-stage scale (modified from Smyth et al., 1990) according to the dissecting results from different populations (Table 1). During stages 1-5, both species had a similar developmental pattern (Fig. 1). The stigma surface levelled with the upper stamens initially (stage 1), but the style grew faster at late stage 2, placing the stigma above all stamens. The upper stamens, however, grew rapidly, levelling with the stigma at stage 3 and then staying above it until the end of stage 6. At stage 7, flowers were pollinated and thus the pistil expanded quickly, inducing the withering of flowers at stage 8. At all stages, the lower stamens never exceeded the height of the stigma.

From stage 3 onwards, the upper stamens of *C. edentula* grew rapidly, overtopping the style and resulting in a dramatically increased distance even after flowering (stage 6). In contrast, the pistil in *C. maritima* seemed to grow more rapidly compared with the upper stamens at later stages, (Fig. 2). 15 sampled buds (60 buds in total for each stage) in *C. maritima* had stigmas that were above the upper stamens at stage 6. Most lower stamens in *C.
maritima stayed below the stigma surface during the whole developmental period, although some of them were recorded as over-topping the stigma at stages 4-6 (Fig. 2).

Anther dehiscence

At early stages, anthers of both species were generally greenish and the two pollen sacs were tightly closed. As anthers became larger, the pollen grains inside the sacs became visible due to the change in the transparency of the pollen sac wall (Ma, 2005). During this period, the anther changed in colour to yellow due to desiccation (Ma, 2005), indicating that the pollen grains were nearly ripe, though the pollen sacs were still intact (pers. obs.). In some cases, small gaps had formed in the wall, but not large enough to allow pollen to be released (e.g., stage 3 of C. edentula in Fig. 3). Finally, the pollen sac ruptured completely and copious amounts of pollen were released.

The timing and the extent to which the pollen was released were very different between the species. In C. maritima, anthers usually released their pollen at late stage 5 (Fig. 3b), while some of them remained closed until the end of stage 6. However, in C. edentula anther dehiscence usually began at early stage 4 (Fig. 3a). When dissecting flowers, no pollen grains were found on the stigma surface in C. maritima until stage 6; in contrast, in C. edentula pollen grains could be found on the stigma surface at early stage 4. Most of the stigmas in C. edentula were covered with pollen grains by the time the flowers opened (Fig. 4a-b); nevertheless, a few stigmas (four flowers from different plants in 60 samples; two from Raspins Beach and two from Sloping Main) were clean on which no pollen shedding occurred even though the flowers were completely open (Fig. 4c-d).

Stigmatic receptivity

Stigmas of both species showed some level of receptivity to pollen at all developmental stages tested (2-6). There were no significant differences of the stigma receptivity between species (P > 0.05), however, stigmatic receptivity in C. maritima increased significantly along with its floral development (Table 2), although no pollen tubes had penetrated the style tract after pollen germination by the time they were fixed (Error! Reference source not found.). In C. edentula, despite the variance between stages, there was no significant change in its stigmatic receptivity: pollen tube growth was observed at all stages, which elongated into the style and even reached the ovules (see Error! Reference source not found.).
Discussion

For a self-fertile plant to be pollinated by another species, or even by other plants of its own species, there needs to be some form of window of opportunity for pollinators to access the stigma before self-pollen fertilises an ovule. Logically, this might involve the timing of anther dehiscence (Ishii & Harder, 2012), pollen release, stigma receptivity (Lankinen et al., 2007) and physical changes in the relative positions of anther and stigma (Takebayashi et al., 2006), all in relation to flower opening. In this study, however, such a pollination window was only obvious between *C. maritima* and a tiny fraction of sampled *C. edentula* flowers.

In both species, anthers dehisced well before the flowers opened, but pollen release only proceeded gradually. Pollen was observed on the stigma in *C. edentula* in most flowers before they opened, whereas this was not usually the case in *C. maritima*. This pattern of anther dehiscence in *C. edentula* differs slightly from previous reports. Meehan (1892) reported that the anthers often dehisce in bud just prior to flowering (i.e., at stage 5), whereas in our study *C. edentula* anthers dehisced earlier (at stage 4). However, instead of pressing stamens against the stigma within the bud to ensure coverage with their own pollen before the flower opens (Meehan, 1892), anthers of *C. edentula* were very close to the stigma but did not have any direct contact with it. Rodman (1974) stated that anthers dehisce introrsely (inwards) in *C. edentula* but somewhat latrorsely in the *C. maritima*. In this study, the anthers in both species were observed to dehisce introrsely.

By the time pollen began to be released, all *C. edentula* stamens had over-topped the stigma surface (whereas in *C. maritima* most short stamens — but not the four long ones — remained below the stigma surface). This placement of anthers above the stigma, interpreted as an effective way to assist self-pollination in self-compatible species, has been referred to as reverse herkogamy (Luo & Widmer, 2013). In *Arabidopsis thaliana*, for example, the outcrossing rate decreased from 10% to 0.3% as a result of increased reverse herkogamy (Luo & Widmer, 2013). Reverse herkogamy has also been argued to be a strategy that maximises pollen uptake and transportation, especially for Lepidopteran pollinators (Kissling & Barrett, 2013).

Ripe pollen from another flower of the same species was able to germinate on a stigma at very early stages of floral development, long before petals opened. This does not necessarily mean that the pollen tube will be able to extend to the ovule, fertilise it and produce viable seeds. For both species, however, stigmas were completely receptive before their flowers had opened. Such observed stigmatic receptivity before flowering has not been reported in

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previous studies (Lankinen et al., 2007). Generally, the stigma of a flowering plant becomes receptive after flowering and thus any compatible pollen can germinate upon deposition; examples of this are *Streptanthus tortuosus* (Preston, 1991) and *Arabidopsis thaliana* (Luo & Widmer, 2013). In some cases, species even show delayed stigma receptivity which reduces the possibility of selfing and promotes outcrossing (Lankinen et al., 2007). The delay period varies between species, from a few hours to 2-3 days (Lankinen & Madjidian, 2011). In this study, we were unable to test whether hand crossing at different stages, especially at early stages, can produce viable seeds since all plants stopped flowering by then. However, it is intriguing to explore whether such early receptivity can lead to the formation of viable seeds in future studies.

Despite our results suggesting that it is most likely that *C. edentula* will be self-pollinated before flowering, not all stigmas in *C. edentula* were contaminated by self-pollen when their flowers opened. A small fraction of dissected flowers in *C. edentula* were completely free from pollen contamination (Fig. 4). The proportion of clean stigmas in *C. edentula* might be even higher in the wild. This is because when dissecting, it is impossible to avoid some self-pollen to fall on to the stigma if the anthers have dehisced. The influence of dissection procedures on pollen release could be inferred in some samples (upper panel in Error! Reference source not found.): (a) instead of pollen being randomly spread over the stigma surface (commonly observed in flowers where pollen was shed naturally; lower panel in Error! Reference source not found.), the contamination sites were located only at the edges of the stigma surface; moreover, (b) the sites where the pollen contamination occurred were exactly where the samples were gently held while buds were dissected. In non-dissected flowers a few exceptions were observed, in which anthers were fully dehisced but the stigma surface was clean without any pollen shedding at stage 6. Thus, Meehan’s (1892) observation that *C. edentula* flowers self-pollinate before flowers open is not always correct. The stigmas of *C. edentula* may be pollinated by pollinators carrying over pollen grains from either conspecific or interspecific plants nearby (e.g., *C. maritima*), thus resulting in a potential pollination window for outcrossing.

These findings suggested that the predominant selfer (*C. edentula*) can be both pollen recipient and pollen donor for inter-specific outcrossing. Such bi-directional hybridisation between predominant selfing species and related species can even be facilitated in the wild. Many self-compatible plants possess physiological mechanisms in the pistil that reduce the performance of self-pollen compared to that of outcross-pollen, including interspecific pollen (Lankinen et al., 2015). Thus, plants could preferentially hybridise when mixed pollen loads are...
applied at similar times despite self-pollen (or intraspecific pollen) shedding on its stigma before alien pollen. This might be the way in which self-fertilisation of *C. edentula* can be circumvented (Donohue, 1998), thus facilitating the high frequency of bi-directional hybridisation in natural populations (Mesgaran et al., 2016): 10 out of 14 sampled populations in our previous studies showed evidence of bi-directional hybridisation (i.e., introgression), with high level of foreign chloroplasts identified (as high as 90% in one population) (Ohadi et al., 2015). Meanwhile, the bi-directional hybridisation might also be modified by environmental effects. These studies were done at a particular time of year, under particular temperatures and humidity and under sheltered conditions. Under different conditions it may be that more — or perhaps fewer — stigmas would have received self-pollen by the time flowers open. Therefore, the relative growth of pollen tubes in reciprocal and mixed applications of pollen, along with a determination of the genotypes of the seeds under pollen competition under different environmental conditions, requires further investigation.

Furthermore, some pollinators may be capable of early pollination, before flowers open. We have not observed nectar robbers entering the sides of buds, but this possibility cannot be ruled out. Visitors to open flowers of both *Cakile* species in southern Australia are most commonly the European honeybee (*Apis mellifera*), large earth bumblebee (*Bombyx terrestris*, only in Tasmania) and cabbage white butterfly (*Pieris rapae*); various native Diptera (especially Syrphidae), Hymenoptera, Coleoptera and Lepidoptera (mostly butterflies) also visit flowers (pers. obs.), while ants, thrips and aphids can commonly be found on plants near to or on inflorescences. Ants in particular were found to be extremely active on experimental plants in the field station (pers. obs.). It is perhaps possible that, during their foraging, these insects could probe into unopened *C. edentula* flowers far enough to brush against the stigma. This could be established using video captured by cameras focusing on developing buds.

Overall, our study showed that there is a window of opportunity for insects facilitating bi-directional hybridisation between related species despite being a predominant selfer and even having contrasting mating systems, which has significant implications on plant colonisation and thus provides novel insights for biosecurity policy makers. Self-incompatible species may suffer severe Allee effects after long-distance dispersal according to Baker’s rule (Baker, 1955). However, bi-directional hybridisation with a related species (presumably the already established self-compatible species, either native or exotic) can provide the newly arriving species with increased mate availability when it spreads into a new environment (Mesgaran et al., 2016), regardless of SI being fully or partially functional in face of interspecific pollen (Kitashiba & Nasrallah, 2014). Since SI is inherited after hybridisation (unpubl. data; also see
Goodwillie & Ness, 2013, Zeng & Cheng, 2014), hybrids and their SI parental species will be more attractive to pollinators, between which pollen flow (gene flow) is more likely to occur (Vekemans et al., 2014). Therefore, after repeated backcrossing, hybrids would be barely distinguishable from the original species (Ohadi et al., 2015). This essentially demonstrates how bi-directional hybridisation provides the newcomer with sufficient mate availability to help ease the Allee effects and promote its establishment (Mesgaran et al., 2016, Bouhours et al., 2017), which might help explain to some extent why there are a great many successful, out-crossing invaders (Snow et al., 2001, Ridley & Ellstrand, 2009).

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Conflicts of interest

The authors declare no conflicts of interest.
References


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Figure legend

**Fig. 1.** Bud stages 1-8 (labelled as S1-S8) photographed under a dissecting microscope in *C. edentula* (top row) and *C. maritima* (bottom row). In *C. maritima*, scale bar = 1 mm at stages 1-3 and 2 mm at stages 4-8; in *C. edentula*, scale bar = 0.5 mm at stage 1, 1 mm at stages 2-5, scale bar = 2 mm at stages 6-8.

**Fig. 2.** Difference in length between the pistil and upper stamens (a, b) and between pistil and lower stamens (c, d) in *C. edentula* and *C. maritima* at different bud developmental stages (see Table 1 for description of these stages). Positive values indicate that the stigma is above the corresponding tallest stamen. The diamond inside the box is the mean while the mid-line shows the median; the whiskers extend between minimum and maximum values.

**Fig. 3.** Anther dehiscence in *C. edentula* (a) and *C. maritima* (b). Scale bars in *C. maritima*, stages 2 = 1 mm, stages 3-6 = 2 mm. Scale bars in *C. edentula*, stage 2 = 1 mm, stages 3 and 5-6 = 0.5 mm, stage 4 = 1 mm.

**Fig. 4.** Pollen shedding onto stigmas at stage 6 in *C. edentula*. Pollen could completely cover the stigma surface (a) or a single area with complete clean stigma elsewhere (b), and even did not shed (c and d) despite fully flowering and anther dehiscence. Scale bar = 2 mm.
Tables

Table 1. Stages of floral development in *Cakile edentula* and *C. maritima* and the landmark events used to define each stage.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Landmark events</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Petals level with long stamens</td>
</tr>
<tr>
<td>2</td>
<td>Petals extend above long stamens</td>
</tr>
<tr>
<td>3</td>
<td>Anther colour changes from green to yellow</td>
</tr>
<tr>
<td>4</td>
<td>Petals colour starts changing from white to pink/purple</td>
</tr>
<tr>
<td>5</td>
<td>Petals extend above sepals and flowers are about to open</td>
</tr>
<tr>
<td>6</td>
<td>Flowers open. The upper stamens keep growing rapidly</td>
</tr>
<tr>
<td>7</td>
<td>Flowers have been pollinated and pistils expand quickly</td>
</tr>
<tr>
<td>8</td>
<td>Anthers wilted and petals have fallen off</td>
</tr>
</tbody>
</table>

Table 2. Comparisons of stigmatic receptivity between stages in both species estimated by odds ratios. Odds ratios less than 1 (e.g., a:b < 1) indicate that the stigmatic receptivity is lower in a than in b. Significant values are in bold (P < 0.05). Estimated odds ratios were based on the higher value of pollen tube growth. For example, stigma receptivity of Stage 2 in *C. edentula* had an estimated odds ratio of < 0.001 compared with that of Stage 6 in *C. maritima*, indicating that the former was more than 1000 times (= 1 / 0.001) likely to have lower receptivity than the latter. Confidence limits including the value “1” indicate the corresponding comparisons are not significant (P > 0.05).

<table>
<thead>
<tr>
<th>Stage 2:3</th>
<th>C. edentula</th>
<th>95% Confidence Limits</th>
<th>C. maritima</th>
<th>95% Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;0.001</td>
<td>(&lt;0.001, &gt;999.999)</td>
<td>0.657</td>
<td>(0.105, 0.705)</td>
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<tr>
<td>Stage 2:4</td>
<td>&lt;0.001</td>
<td>(&lt;0.001, &gt;999.999)</td>
<td>0.055</td>
<td>(0.007, 0.422)</td>
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<tr>
<td>Stage 2:5</td>
<td>&lt;0.001</td>
<td>(0.216, 5.007)</td>
<td>0.004</td>
<td>(&lt;0.001, 0.052)</td>
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<td>Stage 2:6</td>
<td>1.041</td>
<td>(&lt;0.001, &gt;999.999)</td>
<td>&lt;0.001</td>
<td>(&lt;0.001, 0.011)</td>
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<tr>
<td>Stage 3:4</td>
<td>1.905</td>
<td>(&lt;0.001, &gt;999.999)</td>
<td>0.083</td>
<td>(0.011, 0.606)</td>
</tr>
<tr>
<td>Stage 3:5</td>
<td>1.028</td>
<td>(&lt;0.001, &gt;999.999)</td>
<td>0.006</td>
<td>(0.011, 0.071)</td>
</tr>
<tr>
<td>Stage 3:6</td>
<td>&gt;999.999</td>
<td>(&lt;0.001, &gt;999.999)</td>
<td>&lt;0.001</td>
<td>(0.010, 0.701)</td>
</tr>
<tr>
<td>Stage 4:5</td>
<td>0.539</td>
<td>(&lt;0.001, &gt;999.999)</td>
<td>0.068</td>
<td>(0.009, 0.500)</td>
</tr>
<tr>
<td>Stage 4:6</td>
<td>&gt;999.999</td>
<td>(&lt;0.001, &gt;999.999)</td>
<td>&lt;0.001</td>
<td>(&lt;0.001, 0.111)</td>
</tr>
<tr>
<td>Stage 5:6</td>
<td>&gt;999.999</td>
<td>(&lt;0.001, &gt;999.999)</td>
<td>&lt;0.001</td>
<td>(&lt;0.001, 0.213)</td>
</tr>
</tbody>
</table>
Figures

Figure 1:
Figure 2:

C. edentula

Upper stamina
Distance (mm)
Stage

C. maritima

Distance (mm)
Stage

Lower stamina
Distance (mm)
Stage

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Figure 3:
Figure 4:
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Author/s:
Li, C; Cousens, RD; Mesgaran, MB

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