

CONTRIBUTION OF DELAYED AUTONOMOUS SELFING TO REPRODUCTIVE SUCCESS IN *KOSTELETZKYA VIRGINICA*

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ABSTRACT. — *Kosteletzkya virginica* exhibits a delayed autonomous selfing mechanism by stigma lobe curvature. To measure the contribution of delayed selfing to reproductive success in a naturalised population of *K. virginica*, we evaluated the capacity for autonomous self-pollination and compared reproductive success of intact with emasculated open-pollinated flowers. We found no significant differences in the mean number of pollen grains deposited per flower and per stigma between the autonomously pollinated and open-pollinated flowers. There were no significant differences in mean fruit set and in mean number of seeds per capsule between autonomous and hand self-pollination. Fruit set following self-pollination varied significantly depending on the timing of pollination within a day. Emasculatation and pollination treatments did not significantly affect fruit set. In contrast, the mean number of seeds per capsule from intact open-pollinated flowers was higher than that of emasculated open-pollinated flowers. These results indicate that *K. virginica* has a well-developed capacity for delayed autonomous selfing, which increased seed production in the study population.

KEY WORDS. — Malvaceae, *Kosteletzkya virginica*, delayed autonomous selfing, reproductive success, stigma lobe curvature.

INTRODUCTION

Plant pollination mode is related to floral morphology and responds to variation in the surrounding biotic and abiotic environments. In some species, autonomous self-pollination occurs spontaneously within flowers, without an external agent (LLOYD 1979, LLOYD & SCHOEN 1992). For species that rely only on animal vectors for pollen transfer, mechanisms that promote autonomous self-pollination may be of selective advantage in a situation of pollinator limitation (LLOYD 1979).

LLOYD (1979) proposed three modes of selfing: "prior", "competing" and "delayed", depending on the relative timing of selfing and outcrossing. Prior selfing occurs before the receipt of outcross pollen. In competing selfing, self and outcross pollen simultaneously compete for ovules. Both prior and competing selfing provide reproductive assurance. However, they also lower the potential for outcrossing and are subject to pollen and seed discounting (LLOYD & SCHOEN 1992). Delayed selfing occurs after the opportunity for outcross pollen receipt has passed. When delayed selfing is

due to delayed autonomous self-pollination, it is always advantageous, because it assures seed production when pollinators are scarce while allowing outcrossing to dominate when they are abundant (KLIPS & SNOW 1997).

Delayed autonomous selfing may result from different movement mechanisms including (1) pistil (style) movement, such as stigma lobe closure (FETSCHER & KOHN 1999, YANG *et al.* 2004), stigma lobe curvature (BUTTROSE *et al.* 1977, KLIPS & SNOW 1997, RAMSEY *et al.* 2003, RUAN *et al.* 2004a), and style curvature (CULLEY 2002, JESSON & BARRETT 2002, YU & HUANG 2006); (2) stamen (pollen) movement, such as pollen sliding (WANG *et al.* 2004), pollinia bending (DARWIN 1862), anther rotation (LIU *et al.* 2006), stamen curvature (LYON 1992, RATHCKE & REAL 1993) and elongation (ECKERT & SCHAEFER 1998, TRAVESET *et al.* 1998, KALISZ *et al.* 1999, ARMBRUSTER *et al.* 2002, ETCHEVERRY *et al.* 2003); and (3) other floral mechanisms, such as corolla abscission (DOLE 1990, DONNELLY *et al.* 1998), corolla wilting (SUN *et al.* 2005) and corolla closure (RUAN *et al.* 2005a).

Although delayed autonomous selfing has been reported in many plants, there are only few cases in which its impact on reproductive assurance has been tested quantitatively (ECKERT & SCHAEFER 1998, DONNELLY *et al.* 1998, NAGY *et al.* 1999, HERLIHY & ECKERT 2002, KALISZ & VOGLER 2003, KALISZ *et al.* 2004). In some cases, delayed autonomous selfing has been reported to increase reproductive success. The amount of autonomous selfing in *Collinsia verna* varies depending on pollinator conditions (KALISZ & VOGLER 2003), with the amount of selfing increasing with decreasing pollinator abundance. Delayed selfing in *C. verna* provides reproductive assurance (KALISZ *et al.* 2004). Emasculated small flowers produce very few seeds, relative to intact small flowers in *Collinsia parviflora* (ELLE & CARNEY 2003). Under natural pollination, emasculated flowers in *Verbascum thapsus* without delayed selfing experience a reduction in seed set relative to intact flowers with delayed selfing (DONNELLY *et al.* 1998). In contrast, other available studies indicate that autonomous selfing does not substantially increase seed set. KLIPS & SNOW

(1997) observed that autonomous self-pollination in *Hibiscus laevis* did not contribute significantly to fruit production of open-pollinated flowers. By comparing intact with emasculated open-pollinated flowers, NAGY *et al.* (1999) found that autonomous self-pollination in *Kalmia latifolia* did not increase fruit production. In *Aquilegia canadensis*, HERLIHY & ECKERT (2002) found that autonomous selfing increased seed production, but that this benefit was outweighed by the decrease in seed quality as a result of seed discounting and inbreeding depression.

Kosteletzkya virginica L. (Malvaceae), a perennial herbaceous plant, is native to the North American East coast where it grows in salt marshes (GALLAGHER 1985). Each plant produces 1-25 open flowers daily, most of which are hermaphrodite, and each flower lasts only one day (RUAN *et al.* 2005b). Individual plants flower for 25-60 days. Flowers of *K. virginica* have a monadelphous column, surrounded by an exerted pentalobed stigma with synstylous base uniting with the base of the corollas (RUAN *et al.* 2005b). *Kosteletzkya virginica* may be pollinated by insect pollinators or by delayed autonomous self-pollination from stigma lobe curvature (RUAN *et al.* 2005b). If newly opened flowers are not pollinated by insects, then the stigma lobes curve and touch their corresponding anthers before the end of the flower life span (RUAN *et al.* 2005a). Such contact results in delayed autonomous selfing (RUAN *et al.* 2004a, RUAN *et al.* 2005b).

Although the showy, rewarding flowers appear adapted for cross-pollination, *K. virginica* can produce abundant seeds when excluded from pollinators (RUAN *et al.* 2004a). The ratio of flowers displaying delayed autonomous selfing in *K. virginica* is influenced by weather conditions, which influence pollinator behaviour: it is significantly lower on sunny days than on cloudy or rainy days, and also relatively higher in early and late flowering periods (low pollinator abundance and/or activity) compared with the peak of the flowering period (high pollinator abundance and/or activity) (RUAN *et al.* 2005b).

Kosteletzkya virginica was introduced to China in 1993 because of its salt tolerance and potential agriculture uses in marginal soils.

Autonomous self-pollination may be advantageous for species introduced outside their native range where their main pollinators may be lacking. Autonomous self-pollination, if it provides reproductive assurance in the naturalised populations of the species, would be an advantage for agricultural use of *K. virginica*.

In this paper, we evaluate the capacity for delayed autonomous selfing and its contribution to reproductive success in a naturalised population of *K. virginica*. We specifically assessed the capacity of autonomous selfing by: (1) measuring the difference in mean number of pollen grains deposited between the autonomously self-pollinated and open-pollinated flowers; (2) comparing mean fruit set and mean number of seeds per capsule between hand self-pollinated and autonomously self-pollinated flowers; and (3) analysing the effect of timing of selfing on fruit set. We tested the contribution of delayed autonomous selfing to reproductive success by comparing seed production between intact and emasculated open-pollinated flowers.

MATERIALS AND METHODS

This study was conducted from May to September 2006 at the Jinhai Agricultural Experimental Farm located on the Yancheng tideland, 4 km west of the Pacific Ocean (longitude E 119°27'-120°54', latitude N 32°34'-34°28'). Seeds of *K. virginica* from the Halophyte Biotechnology Center (University of Delaware, USA) were planted in the fields in 1993. The population size of naturalised *K. virginica* was approximately 100,000 in 2006. Common and small bees (*Apis*, e.g. *A. cerana* and *A. florae*) are the primary pollinators, but large bees (*Bombus*, e.g., *B. speciosus*), cabbage white butterflies (*Pieris rapae*) and one species of ant (*Formica*) may also serve as pollen vectors (ZHEN *et al.* 2007).

CAPACITY FOR DELAYED AUTONOMOUS SELFING

To measure the difference in the number of pollen grains received by flowers that are open-pollinated and those that only underwent autonomous self-pollination, we established six 1-m² raised-bed plots containing ca. 200 seedlings from the naturalised *K. virginica* population in April 2005. The following spring (2006), each

plot was thinned to 72 individuals, and half of the plots were enclosed by frames constructed of PVC pipe and fibreglass window screen. The screens excluded pollinators and the frames were tall enough to ensure that the screens did not contact the plants. The other half of the plots were accessible to pollinators. Only autonomous self-pollination occurred in the pollinator exclusion plots, while plants in the open plots received pollen delivered by floral visitors (both outcross and self-pollen), in addition to autonomous self-pollination. At 18:00 every day for 26 days, all stigmas of > 30 flowers in each plot were clipped and placed on a slide with 1% acetocarmine solution. Pollen grains adhering to the stigmatic tip were counted under a dissecting microscope (Olympus SZ2-ILST). The mean number of deposited pollen grains of all clipped flowers in each plot represented the pollen grains deposited per flower for each treatment day. The difference in the mean number of pollen grains deposited per flower between the only autonomously self-pollinated and open-pollinated flowers was tested for significance by using a two-sample t-test. To further test for a difference in the mean number of pollen grains deposited per stigma between the autonomously pollinated and open-pollinated flowers, we selected 100 stigmas (one stigma per flower per individual) for the autonomously self-pollinated flowers and 100 stigmas for the open-pollinated flowers, for which pollen grains adhering to the stigmatic tip were counted separately from the other four stigmas within flowers.

We used pollinator exclusion combined with hand-pollination to measure the capacity for delayed autonomous selfing under natural conditions. Forty individuals were arbitrarily selected and tagged in the naturalised *K. virginica* population, and excluded from pollinators with cylinders of coarse wire mesh covered with fine bridal veil. There were two treatments: (1) all stigmas in each flower of 20 individuals were hand self-pollinated between 08:00-09:00; and (2) all stigmas in each flower of 20 individuals were left unpollinated. Fruits were collected just before the mature capsules dehisced, and we counted the number of seeds per capsule. The capacity of autonomous selfing was assessed with a two-sample t-test that compared the mean fruit set and the mean number of seeds per capsule of the flowers between treatments 1 and 2.

The effect of timing of selfing on fruit production was studied by altering the timing of hand self-pollination and measuring the subsequent reproductive success. Seventy plants were arbitrarily selected and tagged in the naturalised *K. virginica* population. During the flowering season, for each selfed plant, half of

the flowers were emasculated and bagged with a nylon mesh before anther dehiscence every day. Every two hours from 07:00 to 19:00 on the day of anthesis, the emasculated flowers of 10 individuals were hand self-pollinated by the pollen from the non-emasculated flowers of the same plant. Previous studies have indicated that stigma receptivity and pollen viability were < 40% at 06:00 and > 50% at 07:00, and that pollen viability was < 30% after 19:00 (RUAN *et al.* unpublished data). The two-hour time intervals were selected to enable systematic evaluation of selfing throughout the day. For each time period, fruit set on each of the 10 individuals was estimated during the flowering season. Differences in mean fruit set among the time periods were tested using the original ANOVA followed by planned comparisons.

CONTRIBUTION OF AUTONOMOUS SELFING TO REPRODUCTIVE SUCCESS

To assess the extent to which autonomous self-fertilisation increases seed set in the study population, we followed the methodology used by ECKERT & SCHAEFER (1998) with a combination of an emasculation treatment and open/controlled pollinations. Eighty pairs of plants were arbitrarily selected in the naturalised *K. virginica* population, with the members of each pair located within 3 m of each other. Daily, two flowers were selected on each plant and matched with two flowers on the paired plant for flowering stage. For one plant in each pair, both flowers were emasculated before anther dehiscence (plant *E*), whereas both flowers on the other plant were left intact (plant *I*). Flowers were emasculated by cutting the anthers from the monadelphous column with a pair of scissors. One of the two flowers on each plant was hand-pollinated with outcross pollen from a third plant (flower *p*), while the other flowers were open pollinated (flower *o*). This results in four treatments: *Eo*, emasculated flowers with open pollination; *Io*, intact flowers with open pollination; *Ep*, emasculated flowers with hand-pollination; *Ip*, intact flowers with hand pollination. Eight hundred flowers were manipulated for each treatment. Fruits were collected when mature, and the number of seeds per capsule was counted.

If autonomous self-pollination increases seed set, then emasculated flowers should set fewer seeds than do intact flowers in the open pollination treatment (seed set: $Eo < Io$). However, two side effects of emasculation could complicate this interpretation: (1) removing anthers could reduce seed set by damaging flowers. The possible negative effects of emasculation on seed set through damage to the flowers can be evaluated by com-

paring *Ep* and *Ip* flowers; and (2) flowers lacking pollen may be less attractive to pollinators so that emasculated flowers set less seed than intact flowers because they receive fewer outcross pollen grains. We do not expect emasculation to reduce the attractiveness of flowers, the reasons for this are not only because the copious nectar (nectar volume per flower is $7.19 \pm 0.12 \mu\text{l}$) produced by flowers of *K. virginica* appears to be the main reward for pollinators, but also because the intact showy crown and yellow monadelphous column after emasculation both are attractive to pollinators. Thus, the emasculated flowers (intact nectar, showy crown and yellow monadelphous column) are potentially as attractive to pollinators as intact flowers. In order to check for differences in pollinator visitation rate between emasculated and non-emasculated flowers, we use data from a previous experiment. In 2005, 6-12 1-m² plots were randomly set up at the field site. At every sampling date, the number of open flowers per plot was recorded, and in half of the plots, the open flowers were emasculated before anther dehiscence. During 20 days across the flowering season, the total number of floral visitors per plot and the number of flowers visited were counted each day from 05:30 to 17:00.

The only other potential problem with this experimental design is that the level of geitonogamous self-pollination (among flowers) and facilitated selfing (within a flower) may be lower for *Eo* than *Io* flowers, because *Eo* flowers have had their anthers removed, whereas *Io* flowers have not. As a result, reduced seed set by *Eo* flowers might be due to reduced geitonogamy and reduced facilitated selfing in addition to the absence of autonomous self-pollination. For *K. virginica*, only two flowers per plant *E* were selected and emasculated every day, but each plant *E* opened 3-30 flowers on each of treatment days (< 5 newly-opened flowers per emasculated plant in a day was observed only three times). Then reduced geitonogamy resulting from emasculation should be negligible. The corollas of *K. virginica* flowers are large (53.11 ± 0.83 mm in diameter), and the main pollinators of *K. virginica* usually visit flowers from the side of flowers, which means that the primary pollinators (common and small bees) usually contact only one of the two floral sex organs. Thus the amount of facilitated selfing due to the activity of pollinators within a flower may be low. Based on this analysis, reduced geitonogamy and facilitated selfing from emasculation do not pose a problem to the interpretation of our results.

We assessed the effects of four treatments (*Eo*, *Io*, *Ep* and *Ip*) on fruit set using the original ANOVA followed by the planned comparisons. We tested the

contribution of autonomous selfing to reproductive success by comparing *Eo* with *Io* and the effect of emasculation damage on reproductive success by comparing *Ep* with *Ip* using planned comparisons (SPSS 11.0). We also tested the difference in pollinator attractiveness between emasculated and non-emasculated flowers by comparing the average visitation time per flower.

RESULTS

CAPACITY FOR DELAYED AUTONOMOUS SELFING

There was no significant difference in the mean number of pollen grains deposited per flower between the only autonomously self-pollinated and open-pollinated flowers (136.5 ± 14.6 vs. 115.1

± 16.5 , respectively; $t = -1.762$, $df = 154$, $P = 0.078$). Single stigmas from the only autonomously self-pollinated flowers received as many pollen grains as open-pollinated flowers (23.77 ± 1.77 vs. 22.08 ± 1.53 , respectively; $t = 0.723$, $df = 198$, $P = 0.471$).

There was no significant difference in mean fruit set between autonomous and hand self-pollination (83.61 ± 0.39 vs. 83.74 ± 0.32 , respectively; $t = -1.530$, $df = 38$, $P = 0.102$). Flowers from which pollinators were excluded set as many seeds as flowers that were self-pollinated by hand (4.28 ± 0.16 vs. 4.31 ± 0.23 , respectively; $t = -1.621$, $df = 38$, $P = 0.083$).

Mean fruit set following hand self-pollination varied significantly among the periods of pollination within a day (Table 1, Fig. 1), but there

Table 1. Effect of timing of selfing on fruit set tested using ANOVA.

<i>ANOVA on data from 7:00-19:00</i>					
Source	Sum of squares	df	Mean square	<i>F</i>	<i>P</i>
Between groups	3444.293	6	574.049	221.949	< 0.001
Within groups	162.943	63	2.586		
Total	3607.236	69			
<i>ANOVA on data from 9:00-17:00</i>					
Source	Sum of squares	df	Mean square	<i>F</i>	<i>P</i>
Between groups	2.007	4	0.502	0.294	0.880
Within groups	76.700	45	1.704		
Total	78.707	49			

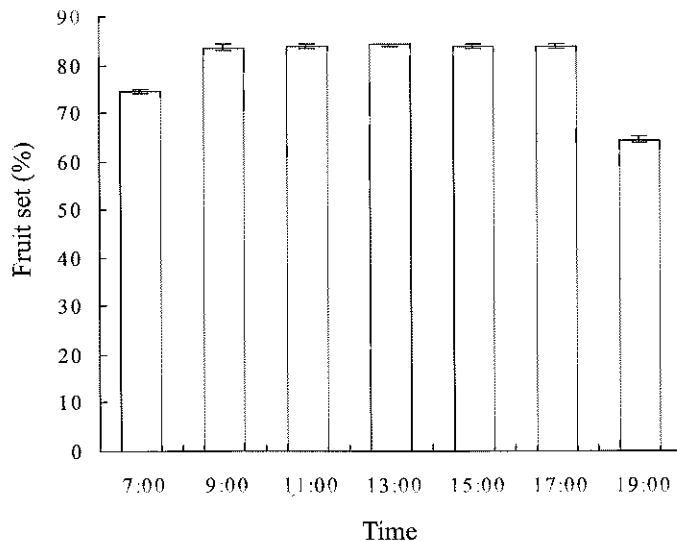


Fig. 1. Effect of timing of selfing on fruit set.

was no significant difference among time periods from 09:00 to 17:00 (Table 1). The planned comparisons indicated that mean fruit sets for the periods 07:00-09:00 ($t_{(67)} = -17.183$, $P < 0.001$) and 17:00-19:00 ($t_{(67)} = -35.610$, $P < 0.001$) were significantly lower than those of the time periods from 09:00 to 17:00, and there was a significant difference between 07:00 and 19:00 ($t_{(63)} = 13.926$, $P < 0.001$).

CONTRIBUTION OF AUTONOMOUS SELFING TO SEED PRODUCTION

Emasculation and pollination treatments did not significantly affect fruit set (Table 2, Fig. 2). There were no significant differences in fruit set between open emasculated flowers (*Eo*) and open intact flowers (*Io*) (planned comparison: $t_{(28)} =$

1.131, $P = 0.267$) and between intact hand-pollinated flowers (*Ip*) and emasculated hand-pollinated flowers (*Ep*) (planned comparison: $t_{(28)} = -0.283$, $P = 0.779$). There was a significant difference in seeds per capsule among the four treatments (Table 3, Fig. 3). To test for a significant contribution of autonomous self-pollination to seed set, we compared the mean number of seeds per flower between open emasculated flowers (*Eo*) (2.11 ± 0.05) and open intact flowers (*Io*) (4.21 ± 0.04), and the difference was highly significant (planned comparison: $t_{(2512)} = -30.863$, $P < 0.001$).

To test for a deleterious effect of emasculating on seed set, we compared mean number of seeds per flower between intact hand-pollinated flowers (*Ip*) (4.30 ± 0.04) and emasculated hand-pollinated flowers (*Ep*) (4.27 ± 0.05), and the difference was not significant (planned comparison:

Table 2. Difference in fruit set among four treatments (*Eo*, *Io*, *Ep* and *Ip*) tested using ANOVA.

Source	Sum of squares	df	Mean square	F	P
Between groups	5.375	3	1.792	0.573	0.637
Within groups	87.500	28	3.125		
Total	92.875	31			

Note. *Eo*: emasculated flowers with open pollination; *Io*: intact flowers with open pollination; *Ep*: emasculated flowers with hand-pollination; *Ip*: intact flowers with hand-pollination.

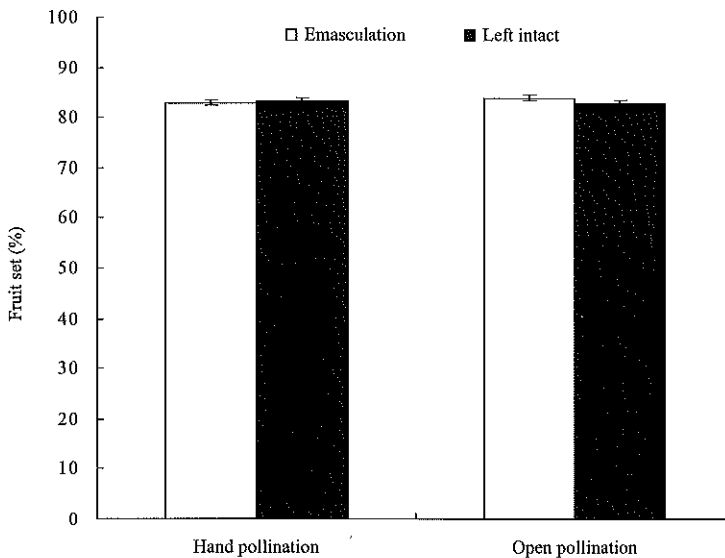


Fig. 2. Effects of emasculating and pollination on fruit set in *Kosteletzkya virginica*. Error bars are ± 1 SE. Analysis of these data is in Table 2.

$t_{(2512)} = -0.464$, $P = 0.632$). This indicates that emasculation does not affect reproductive success of emasculated flowers through physical damage to the flower.

The observed results of pollinator visitation rate showed that there was no significant differ-

ence in the average number of visits per flower between emasculated and non-emascuated flowers (3.38 ± 0.03 versus 3.44 ± 0.06 , respectively; $t = -0.753$, $df = 38$, $P = 0.456$), indicating that emasculation does not reduce the attractiveness of flowers to pollinators.

Table 3. Difference in mean number of seeds per capsule among four treatments (*Eo*, *Io*, *Ep* and *Ip*) tested using ANOVA.

Source	Sum of squares	df	Mean square	F	P
Between groups	2243.803	3	747.934	566.364	< 0.001
Within groups	3317.320	2512	1.321		
Total	5561.122	2515			

Note. *Eo*: emasculated flowers with open pollination; *Io*: intact flowers with open pollination; *Ep*: emasculated flowers with hand-pollination; *Ip*: intact flowers with hand-pollination.

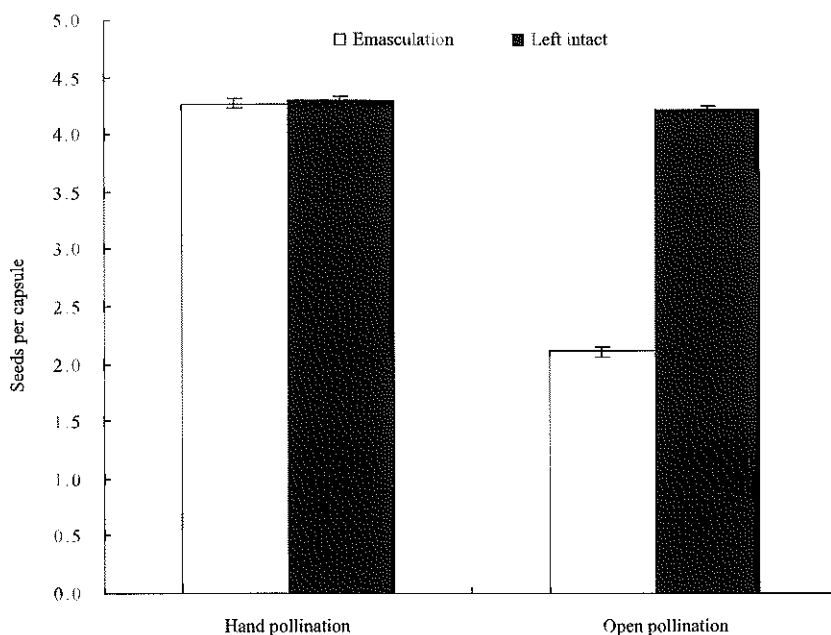


Fig. 3. Effects of emasculation and pollination on seed production in *Kosteletzkya virginica*. Error bars are ± 1 SE. Analysis of these data is in Table 3.

DISCUSSION

In order to demonstrate that delayed autonomous selfing may provide reproductive success in the studied population of *K. virginica*, we should consider at least three conditions: 1) The mechanism of autonomous self-pollination

deposits enough pollen on stigmas; 2) Self pollen is efficient at fertilising ovules; and 3) Insect pollination is limited and autonomous self-pollination increases reproductive success in the study conditions. All those three conditions were validated by our results in the study population during the study period.

First, the pollinator exclusion experiments demonstrated that autonomous self-pollination deposited as many pollen grains on stigmas at the end of the flower life span as open pollination. When delayed autonomous self-pollination occurs, the stigmas touch the anthers on the top part of the monadelphous column. At this time, these anthers have not shed their pollen grains. Such contact could explain why there is no difference in the number of pollen grains per flower between the flowers that were shielded from pollinators and those in the open-pollination treatment. A minimum of 4-6 pollen grains per ovule is generally necessary for maximum seed set (SNOW 1986, MURCIA 1990, SCHUSTER *et al.* 1993). For *K. virginica*, the pollen load per stigmatic surface from autonomous self-pollination amounts to 23.77 ± 1.77 pollen grains, which is sufficient for un-fertilised ovules (even all five ovules) to be fertilised.

Secondly, our experiments with controlled pollination demonstrated that *K. virginica* can self-fertilise both by assisted and autonomous self-pollination, and that the efficiency of delayed selfing is high. In order to assess the level of self-compatibility, a direct comparison of hand self-pollination and hand cross-pollination would be required. This was not the main aim of our study. Nevertheless, evidence for high levels of self-compatibility comes from the indirect comparison of seed set between hand self-pollinated flowers in the assessment of autonomous selfing levels and hand cross-pollinated flowers (*Ep*) in the assessment of autonomous self-pollination contribution to reproductive success. The mean number of seeds was very similar between hand selfed flowers (4.31 ± 0.23) and hand cross-pollinated, emasculated flowers (*Ep*) (4.27 ± 0.05), which indicates that *K. virginica* is fully self-compatible. In addition, we demonstrated that autonomous self-pollination is as successful for seed production as assisted self-pollination. Indeed, there was no difference in fruit set and seed set for flowers that were supplemented with self-pollen and those in which only autonomous self-pollination occurred. In contrast, fruit set was higher in hand-selfed flowers than in the autonomously selfed flowers in *Kalmia latifolia*, indicating that the natural mecha-

nism of selfing is inefficient (NAGY *et al.* 1999). KLIPS & SNOW (1997) made a similar conclusion based on their study of *Hibiscus laevis*.

We found that reproductive success following self-pollination varied among time periods within a day, with lower fruit set early and late in the day. This may result from temporal variation in self-compatibility, stigma receptivity, or pollen viability. In *K. virginica*, stigma receptivity and pollen viability at 07:00 are 59% and 54%, respectively, and pollen viability at 19:00 is only 31% (RUAN *et al.* unpublished data). In contrast, stigma receptivity and pollen viability from 09:00 to 19:00 are over 80% and 50%, respectively (RUAN *et al.* unpublished data), and are likely to be relatively constant, given that we were not able to detect effects of the timing of selfing from 09:00 to 17:00.

Our results indicated that emasculation affected seed set but not fruit set. The fruit capsule of *K. virginica* has five locules, each of which contains a single ovule. For any flower, a fruit capsule containing at least one seed may form after pollination, even if pollination was not sufficient to fertilise all ovules. The non-significant difference in the average visitation rate per flower between emasculated and non-emasculated flowers also indirectly indicates why emasculation did not affect fruit set. In contrast, emasculated flowers without delayed autonomous self-pollination may have fewer chances to fertilise all ovules than non-emasculated flowers with delayed autonomous self-pollination. Stigma pollen loads must be sufficiently high to produce a pollen population effect, i.e. a sufficient number of pollen grains per ovule to trigger germination and/or produce viable pollen tubes (SCHEMSKE & FENSTER 1983, CRUZAN 1986, CRUDEN 2000). For *K. virginica*, after the opportunity to be pollinated by a pollinator has passed and pollination is insufficient to fertilise all ovules, the pollen load from autonomous self-pollination in non-emasculated flowers increases the potential for fertilisation of unfertilised ovules, but not for emasculated flowers.

Finally, in a previous study we showed that delayed autonomous selfing in *K. virginica* directly responds to pollinator environment, the percentage of flowers displaying delayed

autonomous selfing was significantly lower on sunny days than on inclement days (RUAN *et al.* 2005b). This is an additional line of evidence that autonomous self-pollination may increase reproductive success in response to pollinator limitation in the study population of *K. virginica*. Delayed selfing allows outcrossing to occur when pollinators are available, but ensures reproduction and increases plant fitness if pollinators fail to visit flowers, providing that some selfed progeny survive to maturity (KALISZ & VOGLER 2003, KALISZ *et al.* 2004). In *Collinsia verna*, an increase in delayed selfing rates supports reproductive assurance, when the pollination environment in wild populations necessitates reproductive assurance (KALISZ *et al.* 2004). Although delayed autonomous selfing in *Kalmia latifolia* did not contribute to fruit production under natural conditions, it can assure reproduction in the absence of pollinators (NAGY *et al.* 1999) and this reproductive assurance may be important to this species in a year during which pollinators are limiting (RATHCKE & REAL 1993).

The value of selfing as a reproductive strategy depends on the fitness of selfed seeds. For *K. virginica*, although inbreeding depression was 0.54 (SHAN *et al.* 2007), delayed autonomous selfing directly responding to pollinator environment may assure seed production when pollinators are scarce or absent. FISHER (1941) showed that selfing is advantageous from the "automatic selection hypothesis"; an allele for selfing will spread if selfed progeny are at least half as fit as outcrossed progeny (NAGY-LAKI 1976, LLOYD 1979). In contrast, adaptations that allow self-pollination and selfing under variable pollinator environments may be advantageous (KALISZ & VOGLER 2003, KALISZ *et al.* 2004) despite strong inbreeding depression (e.g., 0.64 for *Hibiscus trionum*) (HERLIHY & ECKERT 2002, GOODWILLIE *et al.* 2005, SEED *et al.* 2006). When seeds from autonomous selfing in one flower reduce resources available for potential outcrossed seed in later flowers on the plant, as in the case of *Aquilegia canadensis* (HERLIHY & ECKERT 2002, 2004), seed discounting as a consequence of extreme inbreeding depression drastically reduces the benefit gained through

delayed selfing, obscuring the adaptive significance of autonomous selfing in the populations studied (FENSTER & MARTEN-RODRIGUEZ 2007). Delayed autonomous selfing in *K. virginica* causes no pollen or seed discounting. This is because (1) delayed selfing occurs just before corolla closure, pollen cannot be used by the other flowers after corolla closure; and (2) after corolla closure, unfertilised ovules can not be fertilised from the receipt of outcross pollen.

In conclusion, our results provide evidence that autonomous selfing increases reproductive success in the study population, because emasculated flowers set fewer seeds than intact flowers when open-pollinated. However, future work integrating several types of data such as selfing rates in multiple populations and/or seasons, the contribution of autonomous selfing in different populations, and trade-off between strong inbreeding depression and fitness gain of selfing as the examples of HERLIHY & ECKERT (2002), KALISZ *et al.* (2004) and ECKERT & HERLIHY (2004), will be required to confirm the results of this experiment, and to further provide evidence that the well-developed capacity for autonomous selfing benefits *K. virginica* by providing reproductive assurance. In particular, it should be noted that our study site corresponds to a population introduced outside the native range of the species. Pollinator limitation may be more pronounced in this situation than in native populations.

K. virginica is well-adapted to the coastal tidal flat habitats of China, and it may be used as an agroecoengineering species for alternative agricultural production and for revegetation of salt-affected tidal flats. In addition, the plant has potential use for the production of biodiesel (RUAN *et al.* 2008), with an oil content of 22% in the native lines in American (ISLAM *et al.* 1982), 17.5% in the naturalised lines in China (RUAN *et al.* 2004b), and 20% in selected lines (RUAN *et al.* 2005c). The contribution of delayed autonomous selfing to reproductive success of *K. virginica* indicates that the naturalised populations in China should persist even in the absence of pollination, and provides the potential for exploring the value of the species for salt agriculture.

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