Effects of sib-competition on female reproductive success in Salvia lutescens Koidz. var. crenata

Effects of sib-competition on female reproductive success in *Salvia lutescens* Koidz. var. *crenata*

Kunihiko Takahashi, Takashi T. Makino and Satoki Sakai*

Department of Ecology and Evolutionary Biology, Graduate School of Life Sciences, Tohoku University, Aoba, Sendai 980-8578, Japan

ABSTRACT

Question: Does female reproductive success of a plant become saturated with an increase in seed production due to sib-competition?

Organism: A perennial herb *Salvia lutescens* Koidz. var. *crenata*.

Site of experiments: An experimental garden of Tohoku University, Sendai, northern Honshu, Japan.

Methods: We transplanted 19 parents to the experimental garden after natural pollination. We monitored the survival and flower production of seedlings from those parents for 2 years.

Results: The mean local sib-seedling density increased with an increase in the number of seedlings produced by their parent. The density of sib-seedlings at a distance of 0–1 cm from a focal seedling had negative effects on the survival of the focal seedling. As a result, there was no difference in the number of surviving seedlings between large and small parents in the 2 year period. However, the total number of flowers of the progeny produced by a parent increased linearly with an increase in the number of seeds produced by their parent. Furthermore, the number of flowers of a progeny increased with an increase in the number of seeds produced by its parent, which means that there is a positive relation between the quality of surviving progeny and the number of seeds of its parents.

Conclusion: The female fitness gain curve becomes saturated with an increase in the size of its parent in terms of seedling establishment, but it increases without saturation in terms of flower production by progeny.

Keywords: fitness gain curve, local sib-seedling density, quality of surviving progeny, seedling competition.

INTRODUCTION

The relation between the investment of a plant in female reproduction and its gain in female fitness (female gain curve) is critical in most sex allocation theories, and together with the male gain relation determines the evolutionary stability of a mating system (Charnov, 1982;

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* Author to whom all correspondence should be addressed. e-mail: sakai@mail.tains.tohoku.ac.jp

Brunet, 1992; Klinkhamer *et al.*, 1997; Campbell, 2000). Female fitness has often been assumed to increase at diminishing rates with an increase in the investment in female reproduction (Lloyd and Bawa, 1984; Cheplick, 1992; de Jong and Klinkhamer, 1994). For example, with limited seed dispersal, seed production may intensify sib-competition for resources, resulting in few additional seedlings. Thus, the female fitness of a plant would become saturated with an increase in seed production.

Even though such local resource competition is often proposed as a mechanism that could result in diminishing female returns, few empirical studies have assessed this assumption, and those that have done did not find significant effects of sib-competition on female reproductive success (Casper, 1994; Donohue, 1997; Rademaker and de Jong, 1999). Moreover, few studies have monitored the success of progeny until reproductive maturity, even though survival until the seedling stage does not ensure offspring maturation and reproduction. For instance, size-dependent herbivore attack (Ehrlen, 1995) and the expression of inbreeding depression during later life stages (Dudash and Fenster, 2001) can reduce seedling survival, so that seedling production does not always fully represent female reproductive success.

The purpose of this study was to examine the effects of sib-competition on female reproductive success. We used *Salvia lutescens* Koidz. var. *crenata*, whose seeds are dispersed by gravity without dispersal mechanisms. We monitored dispersed seeds until the flowering stage and examined their flower production. Besides sib-competition, other factors, including differences in micro-environments such as water or nutrients, may affect offspring performance (Keddy, 1981), and it is necessary to exclude these factors when examining the effects of sib-competition. Therefore, we conducted experiments using parents transplanted into a garden where the environmental conditions were nearly uniform.

MATERIALS AND METHODS

Salvia lutescens var. *crenata* is a perennial herb growing on the floor of deciduous or cedar forests in the cool-temperate zone in Japan. Plant height is about 20–80 cm. Each flowering plant produces one raceme with 6–420 flowers that are primarily pollinated by bumblebees. Each flower produces up to four seeds. Seeds have no dispersal system, and hence dispersal distance from the parent is limited. Seeds are dispersed during early autumn, most seeds germinating immediately, and seedlings overwinter as rosettes.

To examine the effects of sib-competition on female reproductive success, we transplanted 19 flowering plants growing naturally on Mt. Kagitori, Miyagi Prefecture, northern Japan (38°15′N, 140°50′E) to an experimental garden at the end of flowering in 2001. These plants had been pollinated naturally and hence there was no artificial limitation on ovule fertilization. Growing conditions in the garden were almost uniform and there was little difference in microhabitats among plants. To reduce seedling competition among non-sibs, the plants were positioned so that their seed dispersal areas did not overlap. The spacing between neighbouring plants was about 30–80 cm. To simulate the forest floor during the growing season, shade cloths were used to reduce light intensity to about 30% that of full sunlight. The garden soil was vermiculite and Kiryu sand. Plants were fertilized with 1/1000 HYPONeX solution (HYPONeX JAPAN Co., Ltd.) every week. Before seed dispersal, we counted the seeds produced by each parent (we could not count the seeds of one of the 19 parents because of their early dispersal). Seeds were dispersed naturally by gravity from the parents and germinated during September 2001. Before winter, the location of each seedling was recorded. During winter, the garden was covered with sheets (Polyester Spunbond,

Unitica Co., Ltd.) to simulate the litter cover that insulates the forest floor during winter. Seedlings that survived developed into flowering or non-flowering (rosette) plants during early summer in 2002. We then counted flowering and non-flowering progeny in September 2002 and 2003. During July 2003, we also counted the flowers produced by each flowering progeny of the 19 transplanted parents.

All analyses were conducted using Stat View 5.0.

Effects of parental seed production on sib-seedling density and survival

We examined the dependence of sib-seedling density on parental seed production and the number of germinated seedlings during 2001. To measure seedling density, we counted seedlings in each of seven circles with radii of 1–7 cm around each seedling. We assessed these local densities for all seedlings produced by each parent and calculated the mean local sib-seedling density for each parent.

We examined at which distance seedlings had an effect on focal seedling survival until September 2002 with multiple logistic regression analysis. By removing the effects of competition with sib-seedlings present at distances less than the distance considered, the effects of competition with sib-seedlings at each distance were detected (e.g. to detect the effects of sib-seedlings at a distance of 2–3 cm, we removed the effects of those at a distance of 0–2 cm).

We also examined the dependence of seedling survival on the local sib-seedling density within a given distance. We classified all seedlings that germinated during 2001 according to whether they survived until September 2002. We analysed the relation of a seedling's survival to local sib-seedling density (D) with logistic regression. Given regression estimates for a and b, the predicted probability of seedling survival (p) is

$$p = e^{a+bD}/1 + e^{a+bD}$$

The logistic regression function is often used to describe binary data as a function of a continuous variable, and a maximum-likelihood method was used to fit a regression curve (Sokal and Rohlf, 1995).

Female fitness gain curve

The female fitness gain curve was obtained from the influence of seed production by maternal parents during 2001 on the number of surviving progeny (including both flowering and non-flowering) and flowering progeny during 2002 and 2003, and the total number of flowers produced during 2003 by all flowering progeny of each parent. Here, non-flowering progeny were less vigorous than flowering progeny, though there was a slight possibility that they could produce flowers after several years. We used an allometric model, $y = ax^b$, to examine these relations, where x is the independent variable, y is the dependent variable (each of the above fitness components), and a and b are constants. Least-squares regression of the natural log-transformed variables was used to estimate a and b. We conducted a single-sample t-test to determine whether b is smaller than 1; $t_s = (b-1)/s_b$, where s_b is the standard error of the regression coefficient (Sokal and Rohlf, 1995). If b is not significantly different from 1, the examined relationship is linear, whereas if b is significantly different from 1, the relationship is decelerating.

Factors affecting flower production by progeny

The number of flowers produced by a flowering progeny could be affected by factors (e.g. genotype) other than environmental factors, including local sib-plant density. Therefore, we examined whether flower production by a flowering progeny during 2003 depended on the mother's seed production after the effects of local sib-seedling competition had been removed. To examine this, we compared flower production among flowering progeny that had no siblings within 0–1, 1–2 . . . 6–7 cm. For example, to remove the effects of sib-competition within 0–1 cm, we compared flower production among progeny that had no siblings within this range.

RESULTS

Seedlings derived from large parents tended to be dispersed over a greater distance than those from small parents, but not significantly so (Fig. 1; Kendall's rank correlation between the number of seeds produced by a parent and the mean dispersal distance of its seedlings, including all 18 parents; $\tau = 0.302$, $n = 18$, $P = 0.0804$).
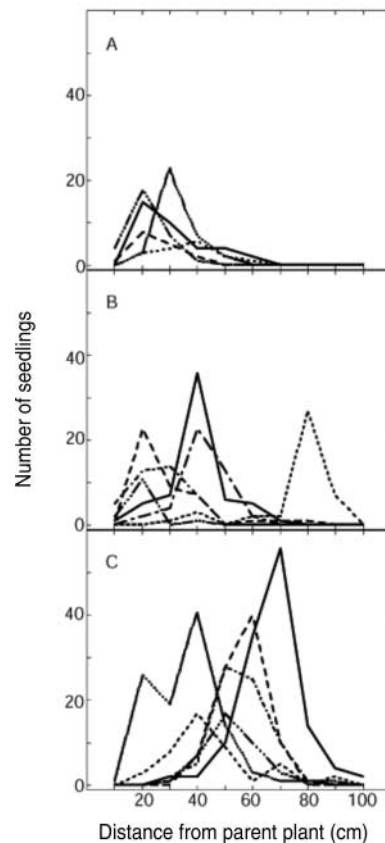

Fig. 1. Frequency distributions of germinated seedlings at different distances from parents that produced 0–90 seeds (A), 90–180 seeds (B) and >180 seeds (C) during September 2001. Different lines represent different parents.

The mean local sib-seedling density within a radius of 1–7 cm varied positively with the mother's seedling production (Table 1; Fig. 2, data of radii of 1, 2 and 3 cm). The mean also tended to vary with the mother's seed production, though this relationship was not statistically significant (Table 1). Thus, seedlings derived from large parents may be exposed to higher sib-seedling competition than those from small parents during the month after their germination.

The probability of seedling survival decreased with an increase in the local sib-seedling density within 1 cm (Table 2 and Fig. 3). Beyond 1 cm, seedling survival did not vary significantly with local sib-seedling density (Table 2).

Table 1. Kendall's coefficient of rank correlation for the relationship between the mean local sib-seedling density for seedlings of a parent to the number of seedlings and seeds produced during 2001 by the parent

Radius (cm)	Number of seedlings (d.f. = 18)		Number of seeds (d.f. = 17)	
	τ	P	τ	P
1	0.450	0.0071	0.112	0.5169
2	0.496	0.0030	0.210	0.2240
3	0.503	0.0026	0.164	0.3405
4	0.496	0.0030	0.236	0.1713
5	0.472	0.0047	0.184	0.2873
6	0.460	0.0059	0.210	0.2240
7	0.413	0.0135	0.210	0.2240

Note: The TPM *P*-value (Zaykin *et al.*, 2002; Neuhäuser, 2004) for the truncation point, 0.05, for the number of seedlings is 3.606×10^{-11}.
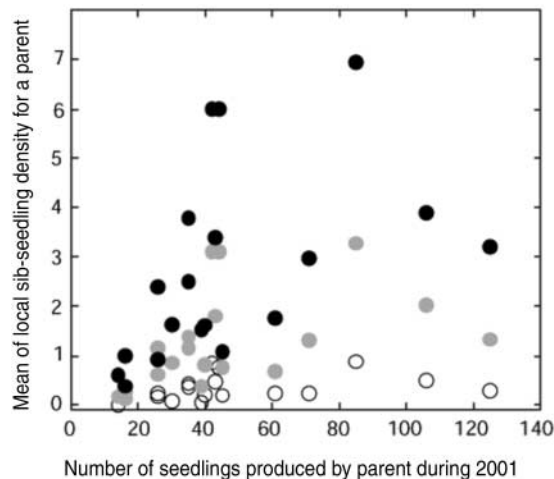

Fig. 2. Relationship between mean sib-seedling density within 1 cm (open circle), 2 cm (grey circle) and 3 cm (solid circle) of seedlings and the mother's seed production during 2001.

Table 2. Adjusted odds ratios for the dependence of seedling survival on its local sib-seedling density at each radius obtained by a multiple logistic regression ($n = 605$)

Radius (cm)	Odds ratio (95% CI)	*P*-value
1	0.710 (0.523–0.962)	0.0272
2	0.968 (0.837–1.120)	0.6634
3	0.974 (0.852–1.115)	0.7066
4	0.957 (0.841–1.088)	0.5003
5	1.001 (0.897–1.117)	0.9850
6	0.918 (0.827–1.019)	0.1080
7	0.892 (0.799–0.996)	0.0421

Note: The TPM *P*-value (Zaykin *et al.*, 2002; Neuhäuser, 2004) for the truncation point, 0.05, is 0.04279.
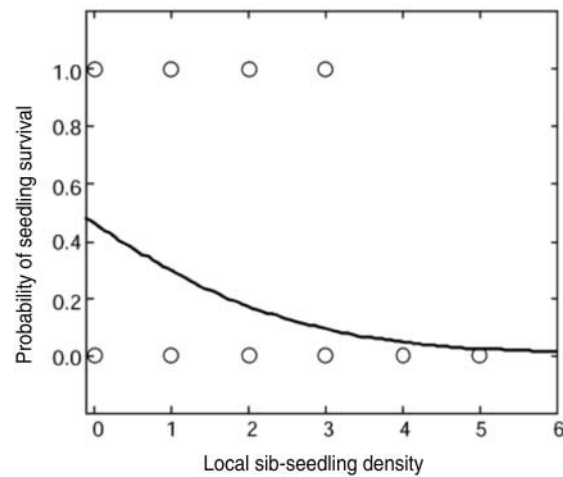

Fig. 3. Dependence of the probability of seedling survival (p) on the local sib-seedling density (within a radius of 1 cm). The curve is based on the maximum-likelihood fit of $p = e^{a + bD}/1 + e^{a + bD}$ ($n = 885$, $\chi^2 = 41.2^*$), where p is the probability of seedling survival and D is the local sib-seedling density ($a = -0.152$ and $b = -0.715^*$). * $P < 0.0001$. Numbers of surviving and dead seedlings are 295 and 347, 53 and 122, 7 and 40, 2 and 14, 0 and 4, and 0 and 1 for local sib-seedling densities of 0, 1, 2, 3, 4, 5 and 6, respectively.

Linear regressions of log-transformed variables revealed that the number of flowering and non-flowering progeny that survived during 2002 and 2003 became saturated with an increase in the number of seeds produced during 2001 by their parent (b was significantly smaller than 1; Table 3 and Fig. 4). Thus, even though large parents produced more seedlings ($y = 19.801 + 0.183x$, $R^2 = 0.376$, $n = 18$, $P = 0.0052$), the number of flowering and non-flowering progeny was not significantly higher than that by small parents. Similarly, the number of flowering progeny during 2003 became saturated with increasing seed production during 2001 by maternal parents (Table 4 and Fig. 4 B), but that during 2002

Table 3. Linear regressions of log-transformed variables

Dependent variable	Overall regression		Test of $b = 1$	
	R^2	P	$b \pm$ S.E.	P
Number of progeny (flowering or non-flowering) that survived during 2002	0.3572	0.0088	0.539 ± 0.181	0.0218
Number of flowering progeny during 2002	0.3053	0.0283	0.569 ± 0.236	0.0788
Number of progeny (flowering or non-flowering) that survived during 2003	0.2902	0.0211	0.419 ± 0.164	0.0028
Number of flowering progeny during 2003	0.3112	0.0161	0.529 ± 0.197	0.0292
Total number of flowers of all flowering progeny during 2003 derived from seedlings produced during 2001 by their parent	0.2160	0.0520	0.651 ± 0.310	0.2053

Note: The independent variable is the number of seeds produced by a parent during 2001 in all regressions.
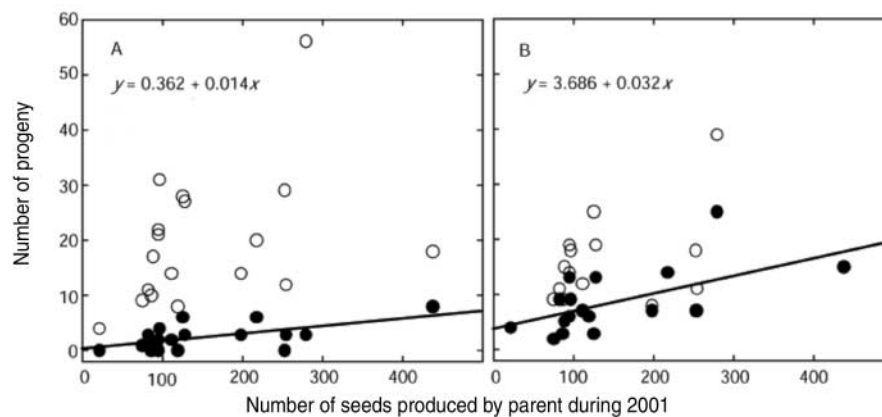

Fig. 4. Dependence of the number of progeny (flowering or non-flowering) that survived (open circle) and the number of flowering progeny (solid circle) during 2002 (A) and 2003 (B) on their parent's seed production during 2001. Linear regressions are for the number of flowering progeny.

increased linearly with increasing seed production during 2001 by maternal parents (b was not significantly smaller than 1; Table 3 and Fig. 4 A). On the other hand, total flower production during 2003 by all sib-flowering progeny of a parent increased linearly with their parent's seed production during 2001 (b was not significantly smaller than 1; Table 3 and Fig. 5). This result suggests that the female fitness gain curve tended to positively increase in a proportional manner in terms of flower production by progeny.

For the progeny with no siblings at distances of 0–1 and 1–2 cm, flowering production during 2003 increased with maternal seed production during 2001 (Fig. 6 and Table 4). This result suggests that the number of flowers of a flowering progeny during 2003 was dependent on the number of flowers of its parent.

Table 4. Dependence of the number of flowers of a flowering progeny in 2003 on the number of flowers produced in 2001 by its parent for progenies which had no sibs in each distance range

Distance (cm)	F-ratio	P	R^2
0–1	16.690	0.00086	0.06392
1–2	9.865	0.00631	0.04632
2–3	3.883	0.06634	0.03382
3–4	1.456	0.24512	0.01463
4–5	0.802	0.38381	0.01796
5–6	0.485	0.49773	0.01570
6–7	0.001	0.96725	0.00005

Note: Weighted regression analysis, d.f. = 1. The TPM *P*-value (Zaykin *et al.*, 2002; Neuhäuser, 2004) for the truncation point, 0.05, is 0.002251.
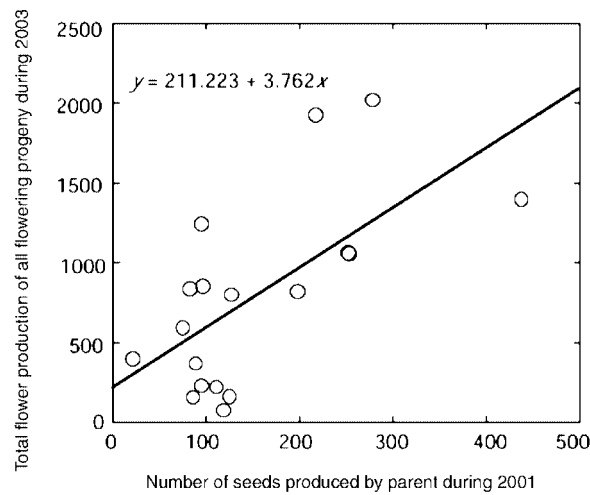

Fig. 5. Dependence of the total number of flowers of all flowering progenies in 2003 derived from the seedlings produced in 2001 by their parent on the number of seeds produced in 2001 by the parent.

DISCUSSION

Seed production and sib-competition

Seedlings derived from large parents experienced higher sib-seedling densities than those from small parents (Fig. 2), even though seedlings derived from large parents tended to be dispersed a greater distance than those from small parents (Fig. 1). In *Salvia lutescens*, seeds have no dispersal mechanisms and are dispersed by gravity. Furthermore, large parents disperse their seeds in one direction since they are often slanting. Thus, the dispersal area is too restricted to reduce sib-competition among sib-seedlings (Table 1). Similar results have
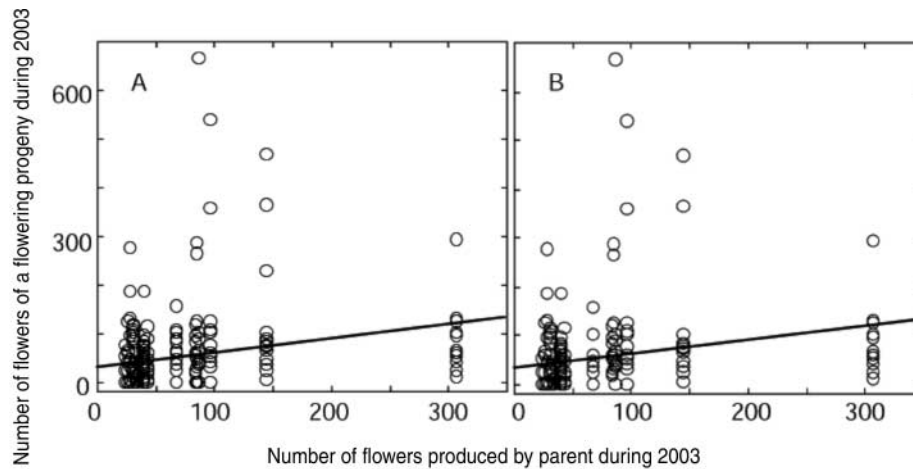
Fig. 6. Dependence of the number of flowers of a flowering progeny in 2003 on the number of flowers produced in 2001 by its parent. (A) Progenies which had no sibs within a radius of 0–1 cm. (B) Progenies which had no sibs within a radius of 1–2 cm. Weighted regressions: (A) $y = 4.92 + 0.05x$, $R^2 = 0.0632$, $P = 0.00086$; (B) $y = 33.69 + 0.29x$, $R^2 = 0.0463$, $P = 0.00631$.

been reported for *Sabatia angularis* (Dudash, 1991) and *Cynoglossum officinale* (Rademaker and de Jong, 1999).

Seedling mortality varied positively with local sib-seedling density (Fig. 3 and Table 2). This result suggests that sib-seedlings growing close to each other compete for limited space or resources, which affects survival, as discussed by Cheplick (1992). Several researchers have reported that competition between individuals may be strong under limited resource conditions, such as water stress, and may be weak under favourable conditions (Keddy, 1981; Tyler and D'Antonio, 1995). However, in our study, seedlings grew under favourable conditions, indicating that competition between sib-seedlings seems to be strong even under such favourable conditions.

Female fitness gain curve

During two years of seedling growth, neither the production of surviving progeny, nor the production of flowering progeny, varied significantly with the seed production of a maternal plant (Fig. 4). This independence may have resulted because mean local sib-seedling density for a parent increased with an increase in the number of germinated seeds produced by the parent (Fig. 2) and because the probability of seedling survival decreased with an increase in its local sib-seedling density (Fig. 3). Thus, at the seedling stage, there were negative effects of sib-competition on female reproductive success in terms of the number of surviving progeny.

However, the total number of flowers of all flowering progeny during 2003 derived from a parent increased with an increase in the number of seeds produced by the parent (Fig. 5). This result suggests that, at the post-seedling stage, the female fitness gain curve was linear rather than convex in terms of flower production by progeny in this experiment.

This result contradicts Charnov's (1982) and Lloyd and Bawa's (1984) prediction that the female fitness gain curve is convex due to the negative effects of sib-competition. This

discrepancy between the female fitness gain curve measured at the seedling and post-seedling stages was due to differences in flower production by progeny. Flowering progeny derived from large parents produced more flowers than those from small parents. The probability of a progeny flowering during 2002 was marginally greater in those derived from large parents than in those from small parents (2002: $\tau = 0.328$; $n = 18$, $P = 0.0574$; 2003: $\tau = 0.229$; $n = 18$, $P = 0.1844$).

Progeny performance until the adult stage is a better measurement of female fitness, so given our findings, we conclude that the effects of sib-competition on female reproductive success in our study were small. However, this might not always be true if there is size-dependent growth inhibition or mortality during the post-seedling stages. If seedlings derived from large parents become larger and suffer stronger damage (e.g. by herbivores) than those from small parents, the advantage of the former seedlings in the post-seedling stage could disappear. Biotic damage may lead to size-dependent negative effects in surviving plants as explained below.

Biotic damage due to herbivores would affect flower production by a flowering plant. Ehrlen (1995) reported the effects of size-dependent herbivore attack on fruit production in *Lathyrus vernus*. The flower buds of our species are often attacked by sawflies in the field (personal observation). If the number of flower buds eaten by sawflies increases disproportionately with an increase in the number of flower buds, the differences in fruit production between large and small progenies would become small. This would also result in the saturation of the female fitness gain curve.

Further analyses are necessary to examine whether these factors exist in *S. lutescens* var. *crenata* and enhance the negative effects of sib-competition on female reproductive success.

Factors affecting flower production by progenies

Excluding the effects of local sib-plant density, flower production by progeny seems to correlate positively with that by their parents. Large parents may produce large seeds that develop into flowering plants of high quality (Sakai and Harada, 2001; Sletvold, 2002; but see Klinkhamer and de Jong, 1987). However, in the species we examined, mean seed weight does not vary with plant size (D^2H) ($R^2 = 0.0105$, $n = 49$, $P = 0.4830$; unpublished field data). In addition, the probability of seed germination is independent of parental size ($R^2 = 0.0099$, $n = 18$, $P = 0.7119$). Thus, the difference in progeny quality was not due to the difference in seed weight. There are thus three possible hypotheses that explain the present results: maternal genetic effects, paternal genetic effects and non-genetic effects.

Progeny quality might increase with an increase in parent size through maternal genetic effects if parents of genetically high quality become large. Several studies have examined the dependence of early seedling performance on maternal genetic effects (e.g. Roach and Wulff, 1987; Kelly, 1993; Weiner *et al.*, 1997; but see Platenkamp and Shaw, 1993), but few have addressed the dependence of late seedling performance (plant size) on maternal genetic effects.

Progeny quality may depend on paternal genetic effects (Marshall and Whittaker, 1989) and may increase with an increase in the size of the mother plant. Large plants can attract more pollinators and receive much more pollen than smaller plants (Geber, 1985; Palmer *et al.*, 1988), resulting in the development of seeds fertilized with higher quality pollen through pollen tube competition (Snow, 1986) or mate choice (Carthew *et al.*, 1996). On the other hand, *S. lutescens* var. *crenata* shows temporal protandry within each flower, avoiding geitonogamy in large plants. Thus, in this species, large parents could obtain pollen of high quality.

There might be no parental genetical effects on progeny quality, and progenies of high quality might be selected through strong sib-competition among seedlings from large parents. That is, the average quality of seeds produced by large parents might be similar to that produced by small parents. However, large parents produce more seeds, although a similar number of seedlings are established than is the case with small parents. Thus, progenies of high quality might be selected during this stage due to strong sib-competition among seedlings from large parents.

Further studies are necessary to examine which factors are involved in the positive correlation between the number of flowers of parents and of progenies.

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