Self- and Cross-Pollination of Encyclia cordigera (Orchidaceae) in Santa Rosa National Park, Costa Rica

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ABSTRACT

The epiphytic deciduous forest orchid *Encyclia cordigera* was manually self-pollinated, out-crossed against one other parent, and out-crossed against a maximum number of other parents in Santa Rosa National Park, Costa Rica. There was no difference in the percent inflorescences that set fruit between selfed and single-parent out-crossed plants, and on the inflorescences that set fruit there was no difference in the percent flowers that bore fruit (85 to 92% for 119 and 82 flowers). However, only 5 percent of the multiple-fathered inflorescences failed to set some fruit, and 97 percent of the flowers set fruit; these values are significantly different from those for the selfed plants and flowers. The overall percent of inflorescences that bore fruit and the percent flowers that bore fruit was very much greater on the hand-pollinated plants than on the unmanipulated ones growing only a few meters away. This finding may indicate that fruit set by *E. cordigera* is pollinator limited, but other interpretations are given.

Is *Encyclia cordigera* (ORCHIDACEAE) SELF-INCOMPATIBLE and does the number of fathers for a plant's fruit crop influence the size of that fruit crop? *E. cordigera* is a common epiphytic orchid in the deciduous forest lowlands of Guanacaste Province, Costa Rica. It is especially common in *Crescentia alata* trees in the flat plateaus in the center of Santa Rosa National Park (SRNP), where this study was conducted. Large numbers of these orchids flower from late February to late March (mid-dry season).

E. cordigera flowers are produced 1-13 per inflorescence, and those on one inflorescence open during a period of about one week. If not pollinated or relieved of pollinia, the flowers last at least 10 days. Once pollinated, the white portions of the petals turn yellow and the ovary begins to swell. In nature, medium- to large-sized black female carpenter bees (*Xylocopa* spp.) enter the flowers in search of nectar and receive the viscidium on the frons or top of the head; the bright yellow pollinia are then later stripped off on the stigma of another flower. The three petals of the flower and the column are colored white and lavender, and shaped such that they are very similar to the flower of *Gliricidia sepium*, a common legume tree that is heavily visited by these same bees for nectar at the time *E. cordigera* is in bloom.

MATERIALS AND METHODS

All orchids were relieved of their pollinaria by sticking the pointed end of a ball-point pen cap or clip into the flower and then withdrawing it with an upward motion. The sticky viscidium readily adhered to the plastic surface. The cap over the four pollinia was knocked off, and in the case of self-pollinated flowers, the pollinia were then placed on the sticky stigma. In outcrossed flowers, the pollinia were carried to another plant and placed on the stigma of a flower that had already been relieved of its pollinaria by the experimenter. Only inflorescences with intact and unpollinated open flowers were used, and all flowers on a test inflorescence were pollinated. Occasionally during transfer one of the four pollinia would fall off, but usually all four pollinia were placed in contact with the stigma.

Outcrossed inflorescences were of two types. "Single father" inflorescences had all their flowers pollinated by pollinia brought from a single other parent (and usually the other parent received the pollinia from the plant that received its pollinia).

"Multiple father" inflorescences had each flower on the inflorescence pollinated by pollinia from a different pollen donor: if there were *n* flowers on the plant, that plant's clutch had *n* fathers. Within 24 hours after pollination, the stigmatic surface of a pollinated *E. cordigera* flower swells to engulf the pollinia and is no longer sticky, so that we have assumed that none of the flowers were subsequently pollinated by bees after the experiment. Flowers of *E. cordigera* do not self-pollinate if the pollinia are not physically moved to the stigma by an outside agent.

The plants were pollinated and marked with pink flagging and orange plastic tags in March 1977. About 90 percent of them were relocated on 12 June 1977 and the green full-sized fruits censused. Apparently owing to severe dry weather during the remainder of the rainy season, almost all of the fruits of *E. cordigera* at this site were aborted between this date and March 1978, and counts of ripe seeds per fruit could not be made. Normally, the fruits of *E. cordigera* release their seeds in March-April, 12-13 months after the flower crop that generated the fruits.

RESULTS

Of 63 inflorescences with self-pollinated flowers 14 (22%) set no fruit, while of 40 single-father inflorescences 6 (15%) set no fruit. On the self-pollinated inflorescences that bore fruit 85 percent (101 of 119) of the flowers produced fruit, while on the single-father inflorescences 92 percent (75 of 82) of the flowers set fruit (table 1). None of these apparent differences in fruit set are statistically significant ($t_{103\ d.f.}=0.91$, n.s.; $t_{199\ d.f.}=1.55$, n.s.). On multiple-father inflorescences, 5 percent (1 of 19) of the inflorescences failed to set any fruit and 97 percent (106 of 109) of the flowers set fruit on fruit-bearing inflorescences. Likewise, these values are not significantly different from their respective contrasts for single-father inflorescences ($t_{59\ d.f.}=1.30$, n.s.; $t_{189\ d.f.}=1.54$, n.s.). However, the multiple-father values are significantly different from the self-pollinated values ($t_{80\ d.f.}=2.01$, $p<0.05$; $t_{226\ d.f.}=3.37$, $p<0.001$). There is a steadily decreasing progression of percent inflorescences that set no fruit and a steadily increasing progression of the mean percent of flowers setting fruit on fruit-bearing inflorescences, in the progression from self-pollinated to multiple-father inflorescences (table 1).

TABLE 1. *Result of self- and cross-pollination of* Encyclia cordigera *flowers.*

	Self-pollinated	Single father	Multiple fathers
Number of inflorescences tested	63	40	19
$\bar{X}$ number of flowers per inflorescence	2.43	2.40	6.05
S.D. number of flowers per inflorescence	1.12	1.52	1.93
Percentage of inflorescences that set no fruit	22	15	5
$\bar{X}$% of flowers setting fruit if fruit was set on inflorescence	85	92	97
S.D.% of flowers setting fruit if fruit was set on inflorescence	21	17	12

If the cross-pollinated flowers are pooled and contrasted with the self-pollinated flowers, statistically different results are not obtained for the percent of the inflorescences that bore no fruits (14% vs. 22%, respectively, $t_{120\ d.f.}=1.531$, n.s.). However, a highly significant greater percent of flowers set fruits on cross-pollinated fruit-bearing inflorescences than on self-pollinated inflorescences ($t_{189\ d.f.}=2.895$, $p<0.01$).

The overall percent of fruit set on the hand-pollinated inflorescences, 70 percent to 91 percent, is enormous compared to that of the unmanipulated orchids growing only a few feet away. On 390 unmanipulated inflorescences scattered among the manipulated inflorescences there were 1297 flower scars and 95 full-sized fruits (7% fruit set). On the fruit-bearing inflorescences in the unmanipulated population, 32 percent of the flowers set fruit; 78 percent of the manipulated inflorescences bore fruit and 85 to 97 percent of the flowers on fruit-bearing inflorescences bore fruit.

The most perplexing result of this experiment was the very high fruit set in all hand-pollinated inflorescences. Among 390 unmanipulated inflorescences there were 2 with four fruits, 2 with three fruits, 18 with two fruits, and 64 with one fruit (one inflorescence outside of the general study area had five fruits). Among the hand-pollinated, fruit-bearing inflorescences, there was 1 with eleven, 3 with seven, 4 with six, 14 with five, 6 with four, 20 with three, 26 with two and 27 with one fruit. It is quite evident that *E. cordigera* can produce much higher clutch sizes than it did in nature in 1977, and than we have seen on these plants in 1976 and 1978.

DISCUSSION

The progression of 22-15-5 percent fruit-less inflorescences from self-pollinated to multiple-fathered plants suggests that much larger samples with the

same experiment might demonstrate some self-incompatibility in *E. cordigera.* Again, the increase in fruit set on fruit-bearing inflorescences over the same progression suggests the same conclusion. While the pooled outcrossed flowers on fruit-bearing inflorescences had a significantly greater fruit set than did the self-pollinated flowers on fruit-bearing inflorescences, the absolute values for both are so high that it is clear that there is only a mild barrier to the development of self-pollinated flowers. Comparison of seed numbers and viability in fruits of different parentage will have to await later experiments.

Janzen (1977) postulated that some optimal number of fathers greater than one, rather than a maximum number of fathers, would constitute the parentage of a flowering plant's clutch. This assumption appears to be true with *E. cordigera* but only barely so, and it seems to make almost no difference whether the single father is maximally closely related or distantly related. However, it is impossible to know if the increased fruit set occurred because there were on average more fathers in the outcrossed set of flowers than in selfed flowers, because the fathers of outcrossed clutches are more distantly related to the mother than are the fathers of self-pollinated clutches, or some blend of both.

A methodological problem needs discussion at this point. The inflorescences of the multiple-fathered inflorescences had about twice as many flowers on them on average as the inflorescences of the self-pollinated and single-fathered inflorescences. The higher percent of inflorescences setting fruit and the higher percent of flowers setting fruit on the multiple-fathered inflorescences might be a reflection of the larger reserves per plant that are suggested by the very large inflorescences. With the experiments we performed, there is no way to examine this possibility. However, we feel that it is not the cause of the differences found because of the trends in fruit set and because of the very high fruit set in all cases of hand pollination.

Since *E. cordigera* will develop many more full-sized fruits per inflorescence if hand-pollinated than occurs naturally, it is tempting to suggest that the plant is pollinator-limited. However, these experiments are inconclusive on this matter. First, natural rates of pollination may always be low and therefore the plant may never have been subject to natural selection for a physiological mechanism to deal with the pollination of more fruits than are appropriate to its overall optimal balance of sexual versus vegetative allocations. In short, a plant that matures four fruits may be running a severe risk of not having enough stored reserves to pass the following dry season(s). Second, it may respond to this unexpected event by not bearing flowers for one or more subsequent flowering seasons, in order to replenish its vegetative reserves. Without conducting this experiment for many consecutive flowering seasons, one cannot know if the plants are pollinator limited. If the plant is not pollinator limited, then we are left with the conclusion that it is, as do many other tropical perennial plants, producing more flowers than mature fruits as an expression of its maleness, for insurance purposes, and to attract pollinators.

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