Crossing systems in *Epidendrum nocturnum* Jacq. (Orchidaceae)

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(Received: April 19. 1985)

Abstract: Unter greenhouse condictions *Epidendrum nocturnum* (Orchidaceae) produced many fruits by self-pollination and cleistogamy. These processes must be optional for the group, since intra- and interspecific pollinations also lead to the formation of fruits having seeds with embryos. The seeds produced by different crossing systems were either without embryos or had 1 to 4. Extra embryos appear more frequently in fruits formed without artificial pollination. On the average, the seeds obtained by artificial cross-pollination germinated more than those removed from naturally formed fruits.

Most orchid species reproduce by allogamy and their flowers show adaptations to cross-pollination. According to Brieger (1966), the constitution of the pollen masses of orchids, as well as the presence of the rostellum, a structure that separates the anther from the cavity of the stigma, cause orchids to be dependent on animals for pollination. According to Dodson (1955), about 80% of all orchid species are pollinated by insects, 3% by hummingbirds, and 15% by different pollinating agents. Only 2% are self-pollinating.

Self-pollination may be an optional or obligate reproductive phenomenon among orchids. Thus, some species that normally reproduce by cross-pollination can be self-pollinated in the absence of their natural pollinating agents (Hagerup, 1952). Other species are cleistogamous, i. e, they are self pollinated before the flowers open. Reports of self-pollination among orchids have been published by Standley (1928), Hagerup (1952), Kirchner (1952) and Knudson (1956).

A few species of orchids reproduce by cleistogamy, as observed by Darwin (1877), Reinchenbach (1977), Forbes (1885), Smith (1905; 1928; 1929), Schlechter (1914), Swamy (1949), Kirchner (1952), and Ilgg (1975).

Contrary to what occurs in most orchid species, which, in greenhouses do not produce fruits, *E. nocturnum* specimens produce many fruits practically throughout the year.

Stort and Martins (1980), in a study of the genus *Cattleya*, observed that self-pollination leads to decreased production of seeds with embryos. Of the fifteen species studied, only *C. aurantiaca*, which normally reproduces by self-pollination, showed higher mean percentages of seeds with embryos when pollinated artificially.

In the present study we attempted to determine the reproduction systems of *E. nocturnum* under greenhouse condictions; we surveyed the production of seeds with and without embryos and determined the germinating power of the seeds produced. The data were compared with those obtained by artificial (manual) pollination.

MATERIAL AND METHODS

The plants of this study were field-collected in Costa Rica and Panama, and kept under greenhouse conditions for several years. On the basis of analysis of the morphological traits and distribution of different *E. nocturnum* populations, Brieger & Bicalho (1978) subdivided this species into groups which the called: *E tridens* Poepp. et Endl., *E. oliganthum* Schltr., *E. bahiense* Reichb. and *E. nocturnum* var. *taguatingense* Brieger e Bicalho and *E. erectum*. In our study we utilized plants of the groups *E. tridens, E. nocturnum* var, *taguatingense* and *E. erectum*. Here we will consider the groups as a single species (*E. nocturnum*), as it was originally considered, and based on the results of experimental hybridizations by Pavanelli and Stort (1985).

After the flowers opened we kept records of visitors to the flowers to determine the presence of pollinating agents. Also, we tried to verify the reproductive systems of the plants. For seed observations we collected ripe fruits obtained by natural pollination (i.e. formed without human intervention), and fruits formed by artificial (intra- and interspecific) pollination and by self-pollination. Artificial pollination was performed manually by transferring a pollinarum of one flower to the stigmatic surface of the same (self-pollination) or another flower (cross-pollination). Crosses were performed between plants from the same region and between plants from different regions, with a total of 218 pollinations. A total of 31 self-pollinations were performed. Five seed samples were removed from each fruit and counted by the tecnique utilized in previous studies (Stort, 1970).

Seeds were tested for germination by sowing samples from each fruit in culture media according to Knudson (1946). Two months after sowing, 10 samples were removed from each medium and 10 slides were prepared. Nine microscopic fields were counted on each slide. The results were compared by the non-parametric Kruskal-Wallis test and by the Mann-Whitney *U* and *Z* tests, according to sample size.

RESULTS

Self-pollination and cleistogamy are the natural processes that lead to fruit formation in the plants studied under greenhouse conditions. No pollinating agent was ever observed on these flowers throughout five years. We noted that the rostellum is more developed in the lateral than in the central portions. This type of formation facilitates self-pollination. A few days after opening of the flower, the pollinia contact the stigma through the central portion of the rostellum that degenerates as the pollen tubes form, while the walls of the column grow towards the central portion, closing it, and the petals and sepals wither and the ovary develops both internally and externally. Internally, megasporogenesis normally occurs, leading to ovule formation. The seeds formed either had no embryo or only 1 to 4 embryos. The embryos are of sexual origin or apomictic(Stort and Pavaneli, 1985).

TABLE I

Percentage average of seeds with embryo obtained by natural and artificial pollination

Species	Natural pollination	Artificial pollinations	
		Self-	Intraspecific
E. nocturnum	59.43	20.92	48.74
E. erectum	28.52	22.28	46.28
E. tridens	44.55	*	71.53
Mean	44.16	21.60	55.52

* No self- pollination

The percentages of seeds with and without embryos and seeds with different numbers of embryos varied according to the type of pollination performed and to the plants involved in the process. In view of the large number of data obtained with the different pollination systems, we present the mean values. Table 1 shows the percentages of seeds with embryos found in fruits formed after natural pollination (including self- and cleistogamy) and after artificial self-and intraspecific pollination.

The *E. nocturnum* plants showed higher percentages of seeds with embryos in naturally formed fruits. In *E. erectum* and *E. tridens* higher percentages were obtained for fruits formed after artificial intraspecific cross-pollination.

Considering the group as a whole (Pavanelli and Stort, 1985), a higher percentage value was obtained for fruits after artificial intraspecific pollination (55,52%) than after self-pollination ($Z = 3.79$, $p < 0{,}05$). The mean percentage of seeds with embryos found after natural pollination (44.16%) was higher than that obtained by artificial self-pollination (21.60%). Statistical comparison of these results yielded $U = 6.00$ (significant , $P < 0{,}05$). On the other hand, the percentage average of seeds with embryos obtained in intraspecific (55.52%) and in interpecific crosses (57.31%) involving the species *E. nocturnum* and *E. erectum* are not statistically significant ($Z = 0.48$). See Table 1.

Numbers of embryos in the seeds. *E. nocturnum* seeds show varying numbers of embryos, as previously indicated. By considering only the seeds with embryos, the following results were obtained for naturally formed fruits: 68.92% seeds with one embryo, 26.36% with 2, 4.35% with 3 and 0.37% seeds with 4 embryos. In seeds formed after artificial intraspecific pollination we found: 91.07% seeds with one embryo, 8.07% with 2, 0.85% with 3 and 0.01% with 4 embryos. Thus, the frequency of 0.01% extranumerary embryos was higher among naturally formed fruits than among those obtained by artificial crossing.

Seed germination: The percentages of germination among seeds obtained by artificial crossing were as follows: *E. nocturnum* 76.80%; *E. erectum*, 89.61% with a mean value of 83.30%. The percentage of germination by crossing *E. nocturnum* and *E. erectum* was 90.67%. Analysis of variance of these results yielded H=7.02, non significant, showing that the seeds resulting from the different crosses did not differ in germinating ability. The seeds removed from naturally formed fruits showed the following germination percentages: *E. erectum* 91.42%; and *E. nocturnum*, 26.51%, with a mean value of 58.96%. Thus, the percentages of germination for seeds obtained by natural process (self-pollination or cleistogamy) were lower than those for seeds formed after intra- and interspecific artificial crossing ($U=8$ $p<0{,}05$).

CONCLUSIONS

E. nocturnum, under greenhouse conditions, produced fruit by self-fertilization and cleistogamy. These processes must be optional for the group, since artificial intraspecific pollination also leads to the formation of fruits having seeds with embryos. The plants also respond to artificial self-pollination.

Naturally formed fruits produce on the average more seeds with embryo than those formed by artificial self-pollination, intra- and interspecific crosses. Similar amounts of seeds with embryos were produced after artificial intra- and interspecific pollination. A lower percentage average of seeds with embryos was obtained in artificial self-pollination.

The seeds produced after the different crossing systems were either without embryos or had 1 to 4 embryos. Seeds with a single embryo were more frequent than those with more than one embryo. Extra embryos appear more frequently in fruits formed without the action of artificial pollination.

The mean percentage of germinability of the seeds obtained by cross-pollination was higher than those from naturally formed fruits. These results may be explained by the fact that the latter exhibit, on the average, higher numbers of aditionad embryos, which besides being smaller than single embryos and therefore less vigorous, suffer competition at the time of germination.

ACKNOWLEDGMENTS

We thank Ernesto Paterniani (Department of Genetics, Escola Superior de Agricultura "Luiz de Queiroz", Piracicaba, USP) for allowing use of the plants. We also thank the Conselho Nacional de Desenvolvimiento Cientifico e Tecnologico, CNPq for financial assistance.

RESUMEN

En condiciones de invernadero, *Epidendrum nocturnum* Jacq. produce muchos frutos mediante autopolinización y cleistogamia. Esos procesos deben ser opcionales para el grupo, pues las polinizaciones intra- e inter específicas también conducen a la formación de frutos que contienen semillas con embrión. Las semillas producidas por los diferentes sistemas de cruzamiento presentan de 1 a 4 embriones. Embriones adicionales aparecen con más frecuencia en frutos de polinización natural. En promedio, la germinación es más exitosa en las semillas producidas por polinización cruzada artificial que en las formadas naturalmente.

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