Impacts of outcross on plant recruitement in *Epipactis*

Daniel PRAT

Université Lyon 1, UMR 5023 LEHNA, 69622 Villeurbanne Cedex, France
daniel.prat@univ-lyon1.fr

Abstract – In many plant species, crosses between related parent leads to inbreeding depression. Few studies have been carried out in orchids on this topic. In *Platanthera*, inbreeding revealed by a reduced growth has been reported. In *Epipactis*, mating systems change from allogamy to autogamy according to the species. This genus is thus suitable to test impacts of mating systems. Selfing, crossing among plants from the same stand or from different stands and interspecific hybridization were tested. Plants of *E. atrorubens*, *E. hellerorine*, *E. muellerii*, *E. palustris* and *E. purpurata* were selfed or crossed. Fruit set was very similar whatever the cross type, including interspecific hybridization. Only a week reduced level of fruit set was observed for crosses among distant plants, which is mostly probably due to an impact of flower transportation and pollinia degradation during conservation. In *E. purpurata* and *E. muellerii*, only selfing was tested and occurred spontaneously. Seeds of *E. purpurata* were not sown. Seeds were introduced between two layers of tissues of 100µm sieve and then buried out in stands with native *Epipactis*. No seed development was observed during the first year. After the second year, some seed plots showed germination and protocorm development. Protocorm development was reported only in few replications. No seed development was reported for *E. muellerii*. In *E. atrorubens* and *E. helleborine*, protocorms with root development were noticed. Most protocorms reached few millimeters and showed occurrence of fungi pelotons. These developments were observed in all type of crosses within species. The lack of development observed in interspecific crosses can be due to the reduced number of replications. Few protocorms in both species showed a larger development with roots up to 1 cm or even more. These larger developments were only observed in seed lots obtained after crosses among plants from distant stands. These results suggest greatly an advantage in plant growth when its result from a cross of genetically different parents. The lack of difference between selfing, crossing among plants of the same stand and spontaneously produced capsules may indicated a large relationships among plants within stands and inbreeding depression. Thus orchid stand management should pay attention to the genetic structure of orchid population in order to preserve it.

INTRODUCTION

Many orchid species are threatened more or less severely by changing environmental conditions and also by genetic isolation due to population fragmentation. Importance of gene flows have been poorly investigated in the context of orchid protection. Studies in population dynamics and plant development revealed impacts of inbreeding like in *Platanthera* (Wallace, 2003). In *Epipactis*, ability to produce seeds after selfing has lready been shown by Tałałaj and Brzosko (2008). In the present study, different types of crosses, including selfing, crosses between plants of the same stand, between plants of distant stands and interspecific hybridization, have been applied on the same plants. Seed development after sowing is then observed in order to detect possible impacts of cross types in some allogamous and autogamous species of *Epipactis*.

MATERIALS AND METHODS

Plant materials: *E. atrorubens*, *E. helleborine*, *E. muelleri* and *E. palustris* plants in native stands, growing under native forest trees or poplar plantation.

Controlled crosses: protecting flower buds by a net (Figure 1), removing pollinarium after flower opening, deposing pollen on stigma for controlled pollination, protecting individual flower or inflorescence by a net (Figure 2) up to mature seed collection. Selfing (in fact geitonogamy), and controlled pollinations have been tested on different flowers of each plant and were identified by color tags. Seeds

Figure 1. Flower buds protected by a net.

produced spontaneously were also included in the study.

Sowing: seeds were extracted from fruits or collected in the protection net, then introduced between two layers of 90 µ sieve tissue (Figure 3) and finally buried into soil close to *Epipactis* plants in late November. Each of the 20 sowing plots included replications (Figure 4).

Epipactis seed development requires several months or years (Tešitelová *et al.*, 2012). Consequently, seeds were collected six months up to two years after sowing and their development was observed under stereoscopic microscope and light microscope.

RESULTS

Fruit set was very similar whatever the cross type; nevertheless pollen degradation could occur during transportation between distant stands inducing a slight decrease of pollination success.

Figure 2. Protection by a net of hand-pollinated flowers.

Figure 3. *Epipactis* seeds on 90 µ sieve tissue during preparation for sowing.

No germination was observed in samples collected 6 months or one year after sowing.

Protocorm development was observed in samples collected two years after sowing for selfing and outcrosses (within and among stands) but not for interspecific hybridization. Only few seed pockets showed germination. No germination was observed for *E. muelleri* (limited number of plots).

Protocorms showed presence of mycorrhizae with hyphae. Large development was observed for *E. atrorubens* and *E.*

helleborine produced by outcross between distant stands (Figure 5).

Figure 4. Plot with several seed pockets before covering with earth.

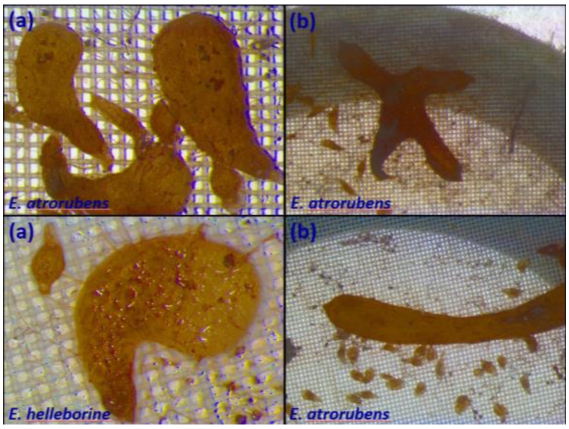

Figure 5. Protocorm development of *E. atrorubens* and *E. helleborine* two years after sowing: **(a)** seeds obtained by spontaneous pollination; **(b)** seeds obtained by crosses between stands.

CONCLUSION

Seeds buried into soil can be still found after two years and showed protocorm development in stands with native *Epipactis* plants but only few developed. Young plants have been observed in *E. atrorubens* and *E. helleborine* but not in *E. muelleri*. The influence of nematodes remains questionable.

Much more important development of protocorms have been observed in seeds derived from interpopulation crosses.

Gene flows among stands could be very important for seed recruitement and should be preserved in stand management for orchid conservation.

Acknowledgements

The present study was initiated with a support of French orchid society and two grants, one from CG38 – Isère, France and one from Botanical society of France

References

Tałałaj I., Brzosko Æ.E. 2008. Selfing potential in *Epipactis palustris*, *E. helleborine* and *E. atrorubens* (Orchidaceae). Plant Syst. Evol., 276: 21–29.

Tešitelová T., Tešitel J., Jersáková J., Ríhová G., Selosse M.A. 2012. Symbiotic germination capability of four *Epipactis* species (Orchidaceae) is broader than expected from adult ecology. Am. J. Bot., 99: 1020–1032.

Wallace L.E. 2003. The cost of inbreeding in *Platanthera leucophaea* (Orchidaceae). *Am. J. Bot.*, 90: 235-242.