REPRODUCTIVE BIOLOGY AND POLLINATION OF THREE ENDANGERED SPECIES OF *HADROLAELIA* (ORCHIDACEAE) IN THE SOUTHEAST OF BRAZIL

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INTRODUÇÃO

1-Introduction

The adaptative ways of reproduction and pollination systems are fundamental to understand plant population dynamics and the features that affect its conservation. Different pollination processes have to been described in Orchidaceae, however it is common observed a very complex system, involving specific pollinator behavior that guarantee the cross - pollination (Passarin and Amaral, 2009). Orchidaceae flowers can offer different kinds of rewards to attract the pollination, such as nectar, oil and floral fragrances (Roberts and Dixon, 2008; Pasarin and Amaral, 2009), but deceptive species, that do not offer any reward, also can be found (Matsui *et al*., 001).

The Orchidaceae family has more than 20,000 described species around the world (Dressler, 2005) and about 2,400 orchids species can be found in Brazil (Barros, 1996). An important Neotropical orchid subtribe is Laeliinae, which support more than 40 genus and almost 1,500 species (van den Berg *et al*., 000). Laeliinae includs many species used as ornamental orchids, such as *Catlleya* and *Laelia*.

The Laneliinae genus *Hadrolaelia* was proposed by Chiron and Castro Neto (2002) after differences observed in species from the genus *Laelia* and *Sophronitis* (van den Berg *et al*., 000), although there is still a discussion about the genus status. *Hadrolaelia* is representing by 22 species, divided in five sections and hybrids can be found in the nature. The geographical distributions of the genus are mainly in the south and southeast of Brazil.

The vegetation ecosystem of Espírito Santo (ES) State, southeast of Brazil, is the Atlantic rain forest and more than 600 orchid species has been registered (Ruschi, 1986). The region of Santa Teresa city, north of ES, is an important reminiscent of this ecosystem, because it has a great species diversity and significant biology importance (Mendes & Padovan, 2000). Three species of *Hadrolaelia* can be found in this region: *H.praestans* (Linden & Rchb.)Chiron & V. P.Castro, *H.pumila* (Hooker)Chiron & V. P. Castro and *H. xanthina* (Lindley) Chiron & V. P. Castro. Although, these species can also be found in other places of the southeast of Brazil, *H. xanthina* and *H.praestans* are considered endangered in all the country and *H. pumila* and *H.praestans* are considered critically endangered in the Espírito Santo State (Simoneli and Fraga, 2007).

OBJETIVOS

In this way, the objective of this work was investigated the phenology, mating systems and pollination mechanisms of this three orchids species, and improve information about its biology and its risks of really extinction.

MATERIAL E MÉTODOS

2 - Materials and Methods

2.1 - Study area and species

Hadrolaelia pumila was studied in the Reserva Biológica Augusto Ruschi (Rebio Augusto Ruschi), ES, southeast of Brazil. There are two well - defined temperature patterns: higher than 22 oC, in January and February, and colder temperatures (3 - 18 oC) in June and July.

The fieldwork of *Hadrolaelia xanthina* and *H.praestans* were carried out in the Estação Biológica de Santa Lúcia (EBSL), ES, which has similar climatic conditions of Rebio Augusto Ruschi. For more details about both study areas see Thomaz and Monteiro (1997) and Mendes and Padovan (2000).

2.2 - Phenology and floral visitors

The studies sites were visited monthly during the years of 2004 and 2005 to phenological observation and daily when plants flowered. Total number of observed individuals was 184 for *Hadrolaelia xanthina*, 325 for *H.praestans* and 385 for *H. pumila*. The reproductive features observed in the field work were: number of flowers in the inflorescence, hour of anthesis, presence of pollinarium, flower colour, flower scent and nectar production.

Observations of flower visitors were made throughout the flowering peak of 2004 and 2005. Observations were made from 06.00 am to 07.00 pm and a total of 160 h/ each species.

2.3 - Reproductive Biology

To define the breeding system of *Hadrolaelia* species, we conducted hand - pollination experiments. These experiments were developed in greenhouse and all the flowers buds produced were bagged with paper bags to avoid visits of pollinators. For *H. pumila*and *H.praestans*, 15 flowers were used to the cross and self - pollination experiments and 20 were used to emasculate and spontaneous autogamy experiments. For *H. xanthina*, 65 flowers were used to cross - pollination experiment, 45 flowers to self - pollination and 80 flowers to spontaneous autogamy experiment. In all these experiments, the inflorescence stayed bagged until ripe fruits were produced. The number of ripe fruits developed was counted for each experiment and seed set was estimated from the proportion of 3,000 seeds with and without embryo produced per fruit, using electronic microscopic. Natural populations used to phenological observation (natural number of flower and fruits produced) were used as control experiment.

All results were expressed as percentage of fruit development and the self incompatibility index were made following Lloyd and Schoen (1992) (% of viable seeds from the self pollination/ % of viable seeds resulted from cross pollinator). In this context, a species is self - compatible when it shows value lower than 0.75. The number of seeds developed by self and cross - pollination were compared using t - test.

RESULTADOS

3-Results and discussion

All three orchids species are epiphytes, however they have different habitats preference in the forest. *Hadrolaelia xanthina* is common in humid and shadow places and its habitats high varies from 12 to 22 m. *Hadrolaelia praestans* habitats high light intensity places. *Hadrolaelia pumila* is found in high varies from 8 to 15 m and places with medium to high lights intensity.

Inflorescence of *H. xanthina* emerged in June, but the flower production started in January. Flowering phenophases occurred for two months, and it finished in the end of February. A total of 65% (N = 184) of the individuals flowering together and produced a maximum of 310 flowers. This species produced from one to seven flowers in terminal inflorescences. These corollas are yellow and the central lobe is white with purple striations. The flowers take about 12 to 24 hours to be completely open. All anthesis period spends from 11 to 14 days and does not have perceptive odors. Nectar production occurred in the sepals base and throughout all anthesis period. The mean nectar volume produced was 9.7 μl (+ 10.9; N = 114).

Flowering phenophase in *Hadrolaelia praestans* occurred from May to October. This species has the longest flowering period, however with lesser flowers production (45 and 53% of individuals flowering in 2004 and 2005, respectively, and produced a maximum of 130 flowers). These flowers corolla are pink - purple, but the inside lobe could be yellow or hard purple. Each pseudobulb usually emitted one flower. The flower takes 12 to 24 hours to be completely opened and the anthesis period varied from 12 to 26 days. A sweet odor is produced throughout the day.

The flowers of *H. pumila* are pink - purple and it spends from 12 to 24 hours to be completely opened. Only one flower is produced by each pseudobulb. The anthesis takes from 10 to 22 days and a sweet smell is produced. Half of the observed individuals (N = 385) flowered both years, producing a maximum of 155 flowers/ year.

Some flowers occurred in very high places, and in this case were impossible observed if pollinarium were collected by the pollinator. *Hadrolaelia xanthina* were the species that had more individuals in this situation (a maximum of 47% of the flowers could not be observed in 2004). *Hadrolaelia praestans* have a maximum of 73.6% of its pollinarium removed, showing a highest frequency among all orchids studied, while *H. xanthina* have the fewest frequencies in both years (maximum of 41%). Fruit production was low in all three species. The maximum registered was: 0.96% in 2005 for *H. xanthina*, 10% in 2005 for *H.praestans* and11% in 2004 for H. pumila.

Interespecific pollination can occurred between *Hadrolaelia praestans* and *H.xanthina*, developing a natural hybrid, called *H. espiritosantensis*, which is described only for ES (Pabst & Dungs, 1975). However, this hybrid was not found in natural habitats throughout the study period.

All three orchids species had a success of 100% (see N above) of the fruits used in the self and cross - pollination experiments. No fruits were developed by agamospermy or spontaneous self - pollination. The autocompatibility index (ISI, sensu Lloyd & Schoen, 1992) shows that *Hadrolalelia xanthina* (0.10%) and *H.praestans* (0.38%) are self - incompatibilities,while *H. pumila* could be considered self - compatibility (1.00%).

The time of fruit maturation varied among the three species, been longest in *H.praestans* (approximately 490 d in self - pollination and 401 d in cross - pollination) than in other species. The time of fruit maturation from self - and cross - pollination was similar in *H. pumila* (approximately 370 d and 360 d, respectively). *Hadrolaelia xanthina* had the smallest time to fruit maturation, been approximately 203 d in the case of self - pollination and 274 d in cross - pollination.

There was no significant ($P > 0.05$) difference between the mean viability of the embryos resulting from self - and cross - pollination in *H. pumila* and *H.praestans*. However,seeds of *H. xanthina* show a significant difference (77.1% seeds with embryo and 31.2% without embryo; t - test = - 19.050, $P < 0.001$).

A similar short flowering period was observed between *H. preastans* and *H. xanthina* in the greenhouse. Intraepecific mating experiments were made with 19 plants and 13 (68.5%) developed fruits. Four of the developed fruits were used to seed viability investigation. The mean number of viable seeds (1247 + 88.4) was similar to the number of seeds without embryo (1752 + 88).

Three species of Hymenoptera were the more frequent visitors observed in *Hadrolaelia xanthina*: *Trigona spinepes* (28 visits), a non - identified bee (15 visits) and *Bombus* sp (one visit). Just one species of wasp was observed and it was recorded in 20 visits. Three hummingbirds species were also observed: *Thalurania glaucopis*, *Ramphodon naevius*, *Clytolema rubricauda*. All of them were recorded just once. The reproductive structures were just touched by *Bombus* sp, however the pollinarium were not removed by any visitor. *Trigona spinepes* and the wasps spend the most of the visit time near the nectarium.

In *H.praestans* it was recorded visits of *Trigona spinepes* and a butterfly species. Both visitors realized seven visits each. In any case the pollinarium was removed or the visitors touched the reproductive structures.

Hadrolaelia pumila were visited by *Trigona spinepes* (two visits), *Apis mellifera* (one visit), and the hummingbird, *Phaetornis eurynome*, which realized the highest number of visits recorded (five). No visitor touched the reproductive structure and the pollinarium were not removed.

Hadrolaelia pumila and *H.praestans* produce a slight fragrance, but produce no nectar or other kind of floral reward. The low fruit production observed seems to be a pattern in Orchidaceae family, especially in the deceptive orchids (Matsui *et al.*, 2001; Pellegrino, *et al.*, 005). The pollination strategy adopted by deceptive orchids is supported by the natural behavior of food exploration realized especially by young bees, which visit many flowers looking for resources (Ackerman, 1986). This behavior guarantees the permanence in a great area and increases the chance of cross pollination (Borba and Braga, 2003), also decreasing the geitonogamy. *Hadrolaelia pumila* and *H. prestans* presents high rates of pollinarium removal, when compared to other deceptive orchids (Borba and Braga, 2003). Furthermore, the flowers of both species are purple that is an important color to take the bees' attraction. The colour associated to the sweet smell produced could stimulate the pollinators' behavior of looking for food.

No effective pollinator was observed removing the pollinarium of the deceptive species studied, although the high percentage of pollinarium that had been removed. Similar results were also observed in others deceptive orchids (Matsui *et al.*, 2001). The manual reproductive experiments indicated that these species are able to developed fruits if all circumstances they need were achieve, as provide in greenhouse conditions. The low fruit set observed in natural condition suggests that these plants could have limitation of the effective pollinator, in spite of the rate of pollinarium removed observed, because only small bees were observed visiting the flowers. It seems that the pollinarium transfer occurred more occasionally than by an effectively pollinator and the low fruit set could reflect it.

Hadrolaelia xanthina produced nectar, offering a nutrional reward to guarantee its pollination, however this species showed a very low fruit set (less than 1%). Some factors could be important to this low success, such as the smallest frequency of pollinarium removed. Moreover, the habitat of *H. xanthina* is shade and at high places in the tree, what favor resource limitation (Montalvo and Ackerman, 1987) and could not support enough energy to fruit develop. Moreover, *H. xanthina* invested differentially in the developed of the seeds formed by self - and cross - pollination. So, it could be another evidence that this species is resource limitated and do not have enough energy to invest in the embryos development if it were resulted of self - pollination and possible have small genetic diversity. In this case, it should be more important guarantee a small fruit set, but the viability of the embryo formed by cross - pollination, than invest in fruits developed by a small genetic variability (Montalvo & Ackerman 1987).

In the study area, *Hadrolaelia espirito - santensis* was not found, although this hybrid was achieved by manual pollination in the greenhouse. It is not common the occurrence of similar flowering period between *H. xanthina* and *H. praestans*, however it could be observed because *H. preastans* emitted its inflorescences in the beginning of February and could open few flowers (pers. obs.), when *H. xanthina* is still flowering. The pollinator which could transfer pollen between these orchids species is *Trigona spinipes*, that is a common visitor of both of them. This small bee is not efficienty in carry out the pollinarium (it was not observed neither once). However, manual mating experiments showed that an half of the seeds of this hybrid could be viable, indicating that if reproduction occurred, more individuals could be found in the area. So, the small coincident flowering periods and the inefficiency of the pollinators seem to be determinant to the small frequency of *H. espirito - santensis* in the EBSL.

CONCLUSÃO

The *Hadrolaelia* species studied show different mating systems: *H. xanthina* and *H.praestans* were self - incompatible, while *H. pumila* was self - compatible.Pollinators seem not to be efficient in transfer the pollinarium in all three species. Despite the high pollinator guild observed, anyone removed the pollinarium and a lower frequency of natural fruit set was observed. *Hadrolaelia pumila* opened many flowers at the same time, what can attract the pollinator and guarantee its visitation. On the other hand, *H. prestans* not produce high flower number, but has a long flowering period, increasing it chances to be pollinated. However the small fruit set observed in the study area could be associated to the absence of an efficient pollinator and indicated that these orchids are pollen limited. It seems that pollinarium is removed occasionally, when pollinator came looking for resources, but it not spend long time in the flower. Moreover, some pollinators are small, like as the frequently *Trigona spinepes*, and not able to carry efficiently the pollinarium. So the pollinarium could be carried out occasionally. (We thank Thiago S. Coser for invaluable help during the field work; the staff of the Reserva Biológica Augusto Ruschi; the staff of the Entomology Laboratory of Universidade Federal de Viçosa for insect identification; CNPq/ Programa Mata Atlântica 690149/01 - 8 for funding; FAPERJ for the post - doctoral fellowship of D.D. Cruz; and CNPq for a productivity grant to T. Wendt).

REFERÊNCIAS

5 - References

Ackerman, J.D. 1986. Mechanisms and evolution of food - deceptive pollination systems in orchids. *Lindleyana* 1: 108 - 113.

Barros, F. 1996. Notas taxonômicas para as espécies brasileiras dos gêneros *Epidendrum, Platystele, Pleurohtallis e Scaphyglottis* (Orchidaeceae). *Acta Bot. Bras.* 10: 193 - 151.

Borba, E.L. & Braga, P.I.. 2003. Biologia reprodutiva de *Pseudolaelia corcovadensis* (Orchidaceae): melitofilia e autocompatibilidade em uma Laeliinae basal. *Rev. Bras. Bot.* 26: 541 - 549.

Chiron, G.R. & Castro Neto, V.P. 2002. Révision dês espèces brésiliènnes du genre *Laelia* Lindley. *Richardiana* 2: 4 - 28.

Dressler, R.L. 2005. Haw many orchid species? *Selbyana* 26: 155 - 158.

Lloyd, D.G. & Schoes, D.J. 1992. Self and cross - fertilization in plants. *Int. J. Plant. Sci.* 153: 358 - 369.

Matsui, K., Ushimaru, A., Fujita, N. 2001. Pollinator limitation in a deceptive orchid, *Pogonia japonica*, on a floating peat mat. *Plant Species Biology* 16: 231 - 235.

Mendes, S.L. & Padovan, M.P. 2000. A Estação Biológica de Santa Lúcia, Santa Teresa: Espírito Santo. *Bol. Mus. Biol. Mello Leitão* 11 - 12: 7 - 34.

Montalvo, A.M. & Ackerman, J.D. 1987. Limitations to fruit production in *Ionopsis utricularioides* (Orchidaceae). *Biotropica* 19: 24 - 31.

Pabst, G.F.J. & Dungs, F. 1975. *Orchidaceae Brasiliensis.* Germany, Hildesheim: Kurt Schmersow.

Passarin, E.R. & Amaral, M.C.E. 2009. Reproductive biology of southeastern Brazilian *Stanhopea* Frost ex Hook (Orchidaceae). *Flora* 204: 238 - 249.

Pellegrino, G., Gargano, D. Noce, M.E., Musacchio, A. 2005. Reproductive biology and pollinator limitation in a deceptive orchid, *Serapias vomeraceae* (Orchidaceae). *Plant Species Biology* 20: 33 - 39.

Robert, D.L & Dixon, K.W. 2008. Orchids. *Current Biology* 18: R325 - R329.

Ruschi, A. 1986. *Orquídeas do Estado do Espírito Santo.* Ed. Expressão e Cultura, Rio de Janeiro.

Simoneli. M. & Fraga, C.N, 2007. *Espécies da flora ameaçadas do Estado do Espírito Santo.* Ipema.

Thomaz, L.D. & Monteiro, R. 1997. Composição florística da mata atlântica de encosta da Estação Biológica de Santa Lúcia, município de Santa Teresa, ES. *Bol. Mus. Biol. Mello Leitão* 7: 3 - 48.

van den Berg, C., Higgins, W.E., Dressler, R.L. *et al.*, 2000. A phylogenetic analysis of Laneliinae (Orchidaceae) based on sequential data from internal transcribed spacers (ITS) of nuclear ribossomal DNA. *Lindleyana* 15: 96 - 114.