

# Phylogenetic relationship between species of the genus *Cattleya* as a function of crossing compatibility\*

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**Abstract:** - The applicability of the graded fertility method in the study of the phylogenetic distance of orchids was investigated with emphasis on the production of  $F_1$  hybrid seeds with embryos. Species of the genus *Cattleya*, subgenera *Monophyllae*, *Aurantiacae* and *Cattleya* were used and the results compared with those obtained with morphological and chemical methods.

A classification should reflect the similarities and differences of the organisms under study. Artificial classifications using one or a few arbitrarily selected traits do not have these characteristics and therefore have no predictive value.

In modern methods of classification, data obtained by analysing traits relating to physiological, cytological, chemical and behavioral properties are complemented by comparative morphological studies and data concerning the geographical distribution of the organisms studied.

Genetical and statistical methods are also very useful for the characterization of taxonomic categories. In general, a genetic method involves the performance of crosses and the comparison of the traits exhibited by the individuals obtained, or of the level of fertility of the crosses effected.

On the basis of experimental data, Clausen *et al.* (1939; 1940; 1945; 1948) and Clausen and Hiesey (1958) suggested the delimitation of specific, and infraspecific categories according to the level of sterility of the hybrids obtained. According to these investigators, this criterion

of grade sterility permits phylogenetic seriation. Dobzhansky (1937; 1957) suggested that gene exchange between the populations studied should be taken into account in species characterization, pointing out that populations of the same species can exchange genes in nature, whereas gene exchange between different species is limited or prevented by the action of specific pollinating agents. Brieger (1960) considered the constancy of genetically fixed distinctive traits protected by mechanisms of reproductive isolation to be important for species characterization. Therefore, the two criteria, the morphological and that based on the level of sterility should not be exclusive, but should be used especially in species characterization. The investigators who accept this approach believe that the great facility in obtaining intergeneric and interspecific hybrids in orchids may be evidence that the mechanisms of reproductive isolation are inefficient or that the taxonomy of the family should be revised.

Working with 24 artificial  $F_1$  orchid hybrids, Stort (1970) found that 17 showed sterility by producing more than 90% of their seeds without embryos. Sterility was complete in 5 hybrids. The degree of sterility of the  $F_1$  hybrids was related to the degree of taxonomic affinity of the species. In the present study using species of the genus *Cattleya*, I attempted

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Subgenus *Monophyllae*

<i>C. autumnalis</i> (Ldl.) Hort.	<i>C. maxima</i> Ldl.
<i>C. chocoensis</i> André	<i>C. mendelii</i> Backl.
<i>C. dowiana</i> Batem.	<i>C. mossiae</i> Hook.
<i>C. gaskelliana</i> Reichb. f.	<i>C. percivaliana</i> (Reichb. f.) O (Brien)
<i>C. gigas</i> Lindl. et André	<i>C. shroederiae</i> (Reichb. f.) Hort.
<i>C. labiata</i> Ldl.	<i>C. trianae</i> Lind. et Reichb. f.
<i>C. lawrenceana</i> Reichb. f.	<i>C. warneri</i> Moore ex Warne.
<i>C. lueddemanniana</i> Reichb. f.	

Subgenus *Aurantiacae*

<i>C. aurantiaca</i> (Batem.) P. N. Don	<i>C. bowringeana</i> Veitch
<i>C. skinneri</i> Batem.	

Subgenus *Cattleya*

Section <i>Intermedia</i>	Section <i>Guttatae</i>	Section <i>Aclandiae</i>
<i>C. forbesii</i> Ldl.	<i>C. amethystoglossa</i> Lind et Reichb. f.	<i>C. aclandiae</i> Ldl.
<i>C. harrisoniana</i> (Batem.)	<i>C. dormaniana</i> Reichb. f.	<i>C. bicolor</i> Ldl.
<i>C. intermedia</i> Graham	<i>C. elongata</i> Rodr.	<i>C. measuresiana</i> Blum.
<i>C. loddigesii</i> Ldl.	<i>C. granulosa</i> Ldl.	<i>C. nobilior</i> Reichb. f.
	<i>C. guttata</i> Ldl.	<i>C. velutina</i> Reichb. f.
	<i>C. leopoldii</i> Verchb. ex. Lem.	<i>C. walkeriana</i> Gardn.
	<i>C. violacea</i> (Kunth) Rolfe	

to estimate the degree of phylogenetic relationship as a function of cross compatibility and emphasized the formation of  $F_1$  seeds without embryos.

## MATERIAL AND METHODS

The species utilized in this study belong to the genus *Cattleya*, according to the taxonomy proposed by Brieger (1960), they are part of the subfamily Epidendroideae, subgenera *Cattleya*, *Aurantiacae* and *Monophyllae*.

The following species were utilized; according to Brieger's classification (1960).

With the exception of *C. bicolor* Ldl., which is tetraploid, ( $2n = 80$ ) all species have  $2n = 40$ , as reported by Blumenschein (1957, 1960, 1961), Kamemoto (1950), Kamemoto and Randolph (1949) and Shimoya (1956).

Artificial crosses were carried out between species of the same subgenus and between species of different subgenera, for a total of 89 different combinations. Only plants with coinciding flowering times were used. All crosses were performed reciprocally and with replications. Ripe fruits were collected and five seed samples taken from each fruit were mounted on slides and stained with Sudan III.

Using a light microscope (125 x), the seeds with and without embryos, encountered in nine microscopic fields per slide, were counted.

## RESULTS

The mean percentages of  $F_1$  seeds with embryos obtained from the different inter-specific combinations are given in Table 1. The combinations involving the subgenus *Monophyllae* produced the following mean percentages of seeds with embryos: *inter se Monophyllae*, 32.50%; *Monophyllae X Intermediae* 49.80%; *Monophyllae X Aclandiae*, 61.26%. The combinations involving species from the *Intermediae* section produced the following mean percentages of seeds with embryos: *inter se Intermediae*, 47.00%, *Intermediae X Aurantiacae*, 42.82%; *Intermediae X Aclandiae*, 45.24% and *Intermediae X Guttatae*, 45.85%. In the crosses involving section *Guttatae*, the mean percentages of seeds with embryos were: *inter se Guttatae*, 50.19%, *Guttatae X Aclandiae*, 46.36%, *Guttatae X Aurantiacae*, 37.95%. Finally, the crosses involving species from the *Aclandiae* section gave the following results: *inter se Aclandiae*, 25.53%; *Aclandiae X Aurantiacae*, 32.72%.

Considering the means of the different

TABLE 1

Results obtained in crosses among different species of the subgenera *Monophyllae*, *Aurantiaceae* and *Cattleya* of the genus *Cattleya*

Sub-genus and section crossed	Percentage of seeds with embryo	Total seeds counted
<i>Monophyllae</i> X <i>Guttatae</i>	40.41	9,915
<i>Monophyllae</i> X <i>Intermediae</i>	49.80	38,318
<i>Monophyllae</i> X <i>Aclandiae</i>	61.26	2,659
X <i>Monophyllae</i> X <i>Cattleya</i>	50.49	
<i>Monophyllae</i> X <i>Monophyllae</i>	32.50	13,282
<i>Intermediae</i> X <i>Intermediae</i>	47.00	14,311
<i>Intermediae</i> X <i>Aclandiae</i>	45.24	19,249
<i>Guttatae</i> X <i>Intermediae</i>	45.85	24,151
<i>Guttatae</i> X <i>Aclandiae</i>	46.36	18,434
<i>Guttatae</i> X <i>Guttatae</i>	50.19	9,057
<i>Aclandiae</i> X <i>Aclandiae</i>	25.53	10,743
X <i>Cattleya</i> X <i>Cattleya</i>	45.02	
<i>Intermediae</i> X <i>Aurantiaceae</i>	42.82	3,884
<i>Guttatae</i> X <i>Aurantiaceae</i>	37.95	1,900
<i>Aclandiae</i> X <i>Aurantiaceae</i>	34.72	5,591
X <i>Cattleya</i> X <i>Aurantiaceae</i>	38.49	
X Genus <i>Cattleya</i>	X 44.32	171,494

values obtained, the crosses between species of the subgenus *Monophyllae* and species of the subgenus *Cattleya* (sections *Intermediae*, *Guttatae* and *Aclandiae*) led to formation of 50.49% seeds with embryos. Crosses between species of the subgenus *Cattleya* yielded 45.02% seeds with embryos and the crosses between species of the subgenus *Cattleya* and species of the subgenus *Aurantiaceae* yielded 38.49% seeds with embryos. The interspecific combinations involving section *Intermediae* with other sections of the genus *Cattleya* (*Guttatae* and *Aclandiae*) led to the formation of 45.55% seeds with embryos.

The overall mean percentage of seeds with embryos from crosses between species of the genus *Cattleya* was 44.22%.

## CONCLUSIONS

Our results show greater affinity between the subgenera *Cattleya* (bifoliate) and *Monophyllae* (monofoliate; 50.49% seeds with embryo) than between *Cattleya* (bifoliate) and *Aurantiaceae* (bifoliate; 38.49% seeds with embryos). These data agree with the results obtained by Tosello (1969) in a study on the flavonol of orchid flowers and show that the

monofoliate and bifoliate characteristics used by Cogniaux (1898) for the separation of taxonomic groups apparently are not indicated, confirming the concept of F. G. Brieger (personal communication).

Within the subgenus *Cattleya*, greater affinity was found between sections *Aclandiae* and *Guttatae* (46.36 seeds with embryos) than between *Intermediae* and *Guttatae*, (45.85% seeds with embryos) confirming the results obtained by Tosello (1969) in his investigation of orchid flavonols. These results do not agree with those obtained by Brieger *et al.* (1963) by the Mahalanobis distance.

Tosello (1969) showed that section *Intermediae* has less affinity with other sections of the subgenus *Cattleya* than has *inter se*. These results were confirmed in the present study, which showed that actually there is a lower affinity between *Intermediae* and *Aclandiae* (45.24% seeds with embryos) and between *Intermediae* and *Guttatae* (45.36% seeds with embryos).

Finally, the *Guttatae* species were shown to have greater affinity for each other, with a higher mean percentage of seeds with embryos (50.19%) than encountered for *inter se* *Intermediae* (47.00%), *inter se* *Monophyllae* (32.50%) and *inter se* *Aclandiae* (25.53%).

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## RESUMEN

La aplicabilidad del grado de fertilidad graduada en el estudio de la distancia filogenética de las orquídeas fue investigada a través de la producción de semillas híbridas F<sub>1</sub> con embrión. Fueron usadas especies del género *Cattleya*, y de los sub-géneros *Monophyllae*, *Aurantiaceae* y *Cattleya*. Nuestros resultados fueron comparados con aquellos obtenidos a través de métodos morfológicos y particularmente por medios estadísticos y cromatográficos usados por otros autores.

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