

Artificial Self-pollination (Autofecundation) as a Taxonomic Tool – *Encyclia phoenicea* (Lindl.) Neumann.

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Abstract

The results of selfing (autofecundation) of plants of *Encyclia phoenicea* (Lindl.) Neumann from Cuba and the Cayman Islands is reported.

This is the second paper of a series that reports the results of over 50 years of selfing (autofecundation) of species by Ruben In Orchids of Miami, Florida. The selfings were made originally to make available to orchid hobbyists what was at the time rare species. In most cases usually only one plant was available of each species, therefore outcrossing was not possible. At the time it was not evident how important the photographic record of the resulting individuals would be.

In the 1950's William Osment of Osment Orchids in Hollywood, Florida introduced into the United States a number of Cuban species that were only known from the literature (Sauleda & Esperon, 2016; Sauleda, 2016). *Encyclia phoenicea* (Lindl.) Neumann, was one of these species. In addition, a seed capsule of *Encyclia phoenicea* from the Cayman Islands was acquired by Marv Ragan of Orange Park, Florida. Accurate photographic records of the results of selfing of plants of *E. phoenicea* from Cuba and the Cayman Islands were kept which now can be used to analyze the introgression history of each species.

Selfing individuals that normally outcross results in a high level of homozygosity due to the expression of recessive alleles in the first generation. Many of the suppressed alleles in a species may be from introgression. Selfing results in the expression of these suppressed alleles giving a glimpse of the evolutionary history of a species.

Ackerman (2014) recognized the heterozygosity of the populations of *E. phoenicea*. He stated: “*Encyclia phoenicea* is a relatively common and highly variable species with considerable ecological amplitude much like populations of *Tolumnia variegata*. It is tempting to describe some of the extreme forms as distinct and there is a current trend to do so. Variation tends to be continuous when examining herbarium specimens across Cuba, but populations in different regions can be distinct both ecologically and morphologically”. The exact nature of the populations is impossible to determine from only examining herbarium specimens without examining live material.

In Llamacho and Larramendi (2005) there is a full page “Illustration showing the variability of *Encyclia phoenicea* flowers”. The illustration clearly demonstrates the introgression with several sympatric species.

Mújica and González (2015) recognize that hybridization occurs with the encyclias in Cuba and state: “After many years of study on this peninsula (Guanahacabibes Peninsula), it is our opinion that in the area, a process of natural hybridization is taking place among *E. plicata*-*E. bocourtii* and *E. phoenicea*-*E. plicata*, species that flower simultaneously in the area and attract the same pollinator”.

Ackerman (2014), Llamacho and Larramendi (2005) and Mújica and González (2015) recognize that hybridization and possible introgression is occurring with *E. phoenicea* in Cuba.

The photographic record made from selfings of *E. phoenicea* demonstrates that introgression has definitely occurred in the Cuban population of *E. phoenicea* but not in the population in the Cayman Islands. In the Cayman Islands the only other encyclia present is *Encyclia kingsii* (C. D. Adams) Nir. a close relative of *Encyclia fucata* (Lindl.) Britt. & Millsp. To date hybrids between these two species has not been found.

In *E. phoenicea* in Cuba there appears to be introgression, but the population in the Cayman Islands does not appear to have hybridized. The question is if what is found in the Cayman Islands is a pure species and one of the parents of *E. phoenicea* in Cuba. However, there are populations in Cuba very similar to the Cayman Island population. Only by selfing these individuals from Cuba that resemble the Cayman Island population can it be determined if what is called *E. phoenicea* in the Cayman Islands is genetically different and still occurs in Cuba.

From the photographic record of the selfings it can be concluded that in Cuba, in *E. phoenicea* there is introgression with whatever species it is sympatric. From the selfings it is easy to see the influence of *Encyclia pyriformis* (Lindl.) Schltr. and *Encyclia plicata* (Lindl.) Britt. & Millsp. The plant that Lindley used to describe *E. phoenicea* (type specimen) may actually be one of the introgression hybrids commonly found. However, without knowing the exact locality of the type, a selfing cannot be made in order to make a determination.

On Isla de Pinos it is clear that *Encyclia xbrevifolia* (Jennings) Ackerman & Mujica is a hybrid of *E. pyriformis* and *E. phoenicea* (Sauleda & Esperon, 2017). There has been introgression in both directions so that in some cases it is difficult to tell the hybrid from the parents without close examination of live material.

Remnant populations of *E. phoenicea* and *E. pyriformis* can still be found in very confined areas on Isla de Pinos. In addition, plants of this introgression hybrid (*E. xbrevifolia*) can be found in flower in almost any season a characteristic of many natural hybrids.

Hybridization in plants especially in the orchidaceae is common (Sauleda & Esperon, 2016). Genome-wide analyses of introgression primarily by backcrossing in plants ranging from oaks to orchids show that a substantial fraction of their genomes are permeable to alleles from related species (Baack et al., 2007). Hybridization can lead to rapid genomic changes which are potentially a creative evolutionary process, allowing genetic novelties to accumulate faster than

through mutation alone (Anderson and Hubricht, 1938; Martinsen et al., 2001). These changes in the genome can lead to rapid selection of beneficial new phenotypes. Mutations are rare, around 10^{-8} to 10^{-9} per generation per base pair (Abbott et al., 2013). Thus, it is likely to take considerable time for novel adaptations to evolve via mutation and natural selection within a species.

Hybridization may contribute to speciation through the formation of new hybrid taxa, whereas introgression of a few loci may promote adaptive divergence and so facilitate speciation (Mallet, 2005). A large amount of introgressed variation can be deleterious, and in most cases hybridization has no impact. However, when large numbers of hybridizations occur among closely related species, such as occurs on the Isla de Pinos with *E. xbrevisfolia*, there is a greater chance that some will result in adaptation and speciation. The Isla de Pinos and *E. xbrevisfolia* are excellent candidates to study adaptive radiation.

On the north coast of Cuba in the Camaguey-Las Tunas provinces *Encyclia xcamagueyensis* a natural hybrid of *E. phoenicea* and *Encyclia altissima* Schltr. is plentiful and appears to be operating as species (Efrain Rodriguez Seijo, pers. comm.). *Encyclia xraganii* Sauleda & Adams a natural hybrid of *E. altissima* and *Encyclia correllii* Sauleda on Andros Island, Bahama Islands is also a large stable population that is reproducing and operating as a species (Sauleda and Adams, 1981). These are prime examples of potential speciation through hybridization.



Encyclia phoenicea (Lindl.) Neumann. Plant from Grand Cayman that was self-pollinated (Autofecundation).



Encyclia phoenicea (Lindl.) Neumann. Labellum of plant originally from Grand Cayman that was self-pollinated (Autofecundation).



Encyclia phoenicea (Lindl.) Neumann. Seedlings resulting from artificial self-pollination (Autofecundation) of a plant from Grand Cayman.



Encyclia phoenicea (Lindl.) Neumann. Seedlings resulting from artificial self-pollination (Autofecundation) of plant from Grand Cayman.



Encyclia phoenicea (Lindl.) Neumann. Plant collected in Cuba by William Osment in the 1950's. Plant that was originally used for artificial self-pollination (Autofecundation).



Encyclia pyriformis (Lindl.) Schltr.



Encyclia phoenicea (Lindl.) Neumann. Seedlings resulting from artificial self-pollination (Autofecundation) of plant from Cuba demonstrating introgression with *E. pyriformis*.



Encyclia plicata (Lindl.) Schltr. Cuban form.



Encyclia phoenicea (Lindl.) Neumann. Seedlings resulting from artificial self-pollination (Autofecundation) of plant from Cuba demonstrating introgression with *Encyclia plicata*.



Encyclia phoenicea (Lindl.) Neumann. Seedlings resulting from artificial self-pollination (Autofecundation) of plant from Cuba demonstrating introgression with *Encyclia plicata*.



Encyclia phoenicea (Lindl.) Neumann. Seedlings resulting from artificial self-pollination (Autofecundation) of plant from Cuba demonstrating introgression.



Encyclia phoenicea (Lindl.) Neumann. Seedlings resulting from artificial self-pollination (Autofecundation) of plant from Cuba demonstrating introgression.



Encyclia phoenicea (Lindl.) Neumann. Seedlings resulting from artificial self-pollination (Autofecundation) of plant from Cuba demonstrating introgression.



Encyclia phoenicea (Lindl.) Neumann. Variation in labellum of seedlings resulting from artificial self-pollination (Autofecundation) of plant from Cuba demonstrating introgression.



Encyclia phoenicea (Lindl.) Neumann. Variation in seedlings resulting from artificial self-pollination (Autofecundation) of plant from Cuba demonstrating introgression.



Encyclia havanensis Bello, Esperon & Saulea. This species is one of the isolated distinct populations of *Encyclia* related to *Encyclia phoenicea* that occurs on isolated tree islands on the north coast of Cuba.



Encyclia xbrevivolia (Jennings) Ackerman & Mujica. Form on Isle de Pinos with flowers that resemble *Encyclia phoenicea* and plant resembling *Encyclia pyriformis*,



Encyclia xbrevivolia (Jennings) Ackerman & Mujica. Form that resembles *Encyclia pyriformis*.



Encyclia xcamagueyensis Rodríguez Seijo, et al. type flower.



First generation artificial hybrid of *Encyclia phoenicea* X *Encyclia plicata*. Flowers demonstrate fleshy pad on labellum typical of *Encyclia plicata*.



First generation artificial hybrid of *Encyclia phoenicea* X *Encyclia pyriformis*. Flowers have typical striping on labellum of *Encyclia pyriformis*.

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