THE PRICKLY PROBLEM OF INTERWOVEN LINEAGES: HYBRIDIZATION PROCESSES IN CACTACEAE

EL ESPIOSO PROBLEMA DE LOS LINAJES ENTRECruzADOS: PROCESOS DE HIBRIDACIÓN EN CACTACEAE

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Abstract
Background: Hybridization in nature occurs in numerous botanical families. In particular, the Cactaceae family contains lots of genera in which hybridization is reported.

Questions: What are the patterns of reported natural hybridization in Cactaceae and their probable causes? Are there phylogenetic and evolutionary implications related to hybridization, particularly in Opuntioideae?

Data description: A total of 62 articles about natural hybridization and classical Cactaceae literature were reviewed.

Study site and dates: From 1900 to June 2021

Methods: A search for articles was performed in Web of Science and Google Scholar with the keywords “Cactaceae hybridization”, for time span “1900 to 2021” and included information from classic family-specific monographs.

Results: Natural hybrids in Cactaceae occur in subfamilies, Cactoideae and Opuntioideae. There is evidence of nonselective mechanisms of reproductive isolation, but only for few taxa. For Cactoideae members the main approach used was morphological description, and the tribe with the highest number of natural hybrids was Trichocereeae. In Opuntioideae, the reviewed articles performed mostly chromosome counts, morphometric and phylogenetic analyses, and showed the highest number of natural hybrids.

Conclusions: It has been suggested that hybridization impacts the evolution of Cactoideae and Opuntioideae, but few studies have formally tested this hypothesis. In Cactoideae, we found only descriptive evidences of hybridization; therefore, previous statements suggesting an important role of hybridization in the evolution of Cactoideae should be supported by performing formal analyses. For the postulation that hybridization impacts the evolution of Opuntioideae, we found formal evidence supporting hybridization hypothesis unlike what we found in Cactoideae.

Keywords: Discordant phylogenies, natural hybridization, Opuntioideae, reticulate evolution, speciation

Resumen
Antecedentes: La hibridación natural se presenta en numerosas familias botánicas. En particular, la familia Cactaceae presenta muchos géneros con registros de hibridación.

Preguntas: ¿Cuáles son los patrones reportados de hibridación natural en Cactaceae y sus probables causas? ¿Existen implicaciones filogenéticas y evolutivas relacionadas con la hibridación, particularmente en Opuntioideae?

Descripción de datos: Se revisaron 62 artículos sobre hibridación natural y literatura clásica de Cactaceae.

Sitio y años de estudio: De 1900 a junio de 2021

Métodos: Una búsqueda en Web of Science y Google Scholar con las palabras clave “Cactaceae hybridization”, un lapso de tiempo de “1900 a 2021”, así como información de monografías clásicas.

Resultados: Los híbridos naturales en Cactaceae se encuentran en Cactoideae y Opuntioideae. Hay evidencia de mecanismos no selectivos de aislamiento reproductivo, pero solo para pocos taxones. En Cactoideae el enfoque principal fue la descripción morfológica, y la tribu con más híbridos naturales fue Trichocereeae. En Opuntioideae, los artículos revisados realizaron principalmente conteos cromosómicos, análisis morfométricos y filogenéticos, y mostraron el mayor número de híbridos naturales.

Conclusiones: Se había sugerido que la hibridación impacta la evolución de Cactoideae y Opuntioideae, pero pocos estudios han probado formalmente esta hipótesis. En Cactoideae, encontramos evidencias descriptivas de hibridación; por lo tanto, las declaraciones previas que sugieren un papel importante de la hibridación en la evolución de Cactoideae deben ser apoyadas mediante análisis formales. Para la postulación de que la hibridación impacta la evolución de Opuntioideae, encontramos evidencia formal que apoya las hipótesis de hibridación diferente de lo encontrado en Cactoideae.

Palabras clave: Filogenias discordantes, especiación, evolución reticulada, hibridación natural, Opuntioideae.
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Hybridization is a process that occurs in plants from ferns to angiosperms (Whitney et al. 2010) and is considered a process that impacts the speciation and evolution of the groups in which it is present (Anderson 1953, Arnold 2004, Soltis & Soltis 2009, Soltis 2013). It is a natural and common process in numerous families, with some classic examples of hybridization occurring in Asteraceae (Helianthus), Cactaceae (Opuntia), Fagaceae (Quercus), Iridaceae (Iris), Pinaceae (Pinus) and Rosaceae (Lachemilla, Rosa) (Critchfield 1986, Rieseberg 1991, Jensen et al. 1993, Pinkava 2002, Arnold 2006, Meng et al. 2011, Morales-Briones et al. 2018). Although numerous studies have been conducted in these families, it is still difficult to fully understand the dynamics and outcomes of hybridization in these groups.

Hybridization has been defined as the mixture of two distinct lineages and can occur between different species of the same or different genera (Arnold 1997). Genetic exchange can occur between individuals from different populations or from lineages belonging to any taxonomic category; the result of these crosses is known as a hybrid (Arnold 1997, Soltis 2013). Genetic exchange can occur by artificial or natural crossing; when spontaneous hybridization occurs in nature without anthropogenic intervention, it is known as natural hybridization (Arnold 1997).

Cactaceae has approximately 1,438 to 1,851 species distributed in four subfamilies, Pereskioideae Maihuenioideae, Cactoideae and Opuntioideae, with the last two being the most diverse and containing approximately 1,221 and 176 species, respectively (Hunt et al. 2006, Korotkova et al. 2021). Members of Cactaceae exhibit a high number of evolutionary novelties related to their association with arid environments, such as crassulacean acid metabolism (CAM), the presence of waxes and trichomes (Anderson 2001), and a wide range of growth forms, such as globular, globose-depressed, cylindrical, columnar and articulated (Vázquez-Sánchez et al. 2012).

In particular, the family Cactaceae has numerous genera in which natural hybridization has been reported in classical taxonomic reviews and some recent studies (Anderson 2001, Pinkava 2002, Hunt et al. 2006, Machado 2008); this hybridization mainly occurs in the subfamilies Cactoideae and Opuntioideae. Cactoideae (Figure 1A, B) has the most attractive genera in the family, making them widely studied in terms of the description of new genera and species based mainly on morphological characters. Infrequent hybridization has been found among members of this subfamily, and the impact of these hybrids on the evolution of the species has been poorly studied; furthermore, there are also few published works focused on demonstrating the existence of these hybrids (Machado 2008).

Opuntioideae is known for its areoles with glochids, an articulated growth pattern that is related to vegetative dispersion, and orbicular seeds covered by a bony aril (Anderson 2001) (Figure 1B). The morphological characteristics that have allowed this group to adapt to desert environment conditions also make its members difficult to preserve in biological collections (Pinkava 2002, Majure et al. 2012b), although in Mexico, the National Herbarium (MEXU) has a high number of records for Cactaceae (more than 21,000), particularly for Opuntia (9,259). Finally, in Opuntioideae, hybridization has been comparatively better studied, which, along with polyploidy, has been considered to play a role in the evolution of its species (Pinkava 2002).

Phylogenetic analyses carried out in the Cactaceae subfamilies have revealed that some traditional classifications at the generic level are not representative of real phylogenetic relationships between species considered to belong to certain genera. Some examples include phylogenetic analyses carried out in the tribes Cactae (Vázquez-Sánchez et al. 2013) and Hylocereae (Korotkova et al. 2017), as well as the genera Astrophytum (Vázquez-Lobo et al. 2015), Cephalocereus (Tapia et al. 2017), Opuntia (Majure et al. 2012b) and Pereskia (Butterworth & Wallace 2005). Phylogenetic relationships for the above mentioned tribes and genera are better understood, but there are still other groups in this family in which relationships at the genus or species level have not been resolved due to processes such as incomplete lineage sorting (ILS) or hybridization (Majure et al. 2012b, Copetti et al. 2017, Granados-Aguilar et al. 2021).

It has been proposed that hybridization is a fundamental part of the evolutionary process in Cactaceae, so our objective was to analyze in which genera natural hybridization has been reported, its probable causes, and the phylogenetic and evolutionary implications it has on the groups where it is present, emphasizing the case of Opuntioideae. A previous hybridization review (Machado 2008) summarizes examples of putative hybrids in Cactoideae, but for such a complicated process, it is necessary to perform a systematic qualitative review of the literature to better un-
Understand the past and current status of this process, allowing us to advance the study of hybridization. The particular objectives of the current review are I) to carry out a literature review of natural hybrids reported for Cactaceae in classical monographs and scientific articles, II) to briefly summarize examples of prezygotic and postzygotic barriers in Cactaceae, III) to evaluate the evidence of the role that natural hybridization plays in the evolution of Cactaceae subfamilies, and IV) to identify if there is evidence of unresolved or discordant phylogenetic histories due to hybridization in Opuntioideae.

**Methods**

We used the classic family monographs by Anderson (2001) and Hunt *et al.* (2006) as an information source, and we performed a search on the Web of Science in June of 2021 with the keywords “Cactaceae hybridization”; for time span, we selected “1900 to 2021”. From the 72 articles obtained in the search, 18 were excluded because they only used some of the keywords, but their main topic was not about hybridization in Cactaceae. In addition, a search was conducted in Google Scholar with the same search criteria, and eight other articles were found. Table S1 shows the integration of the information collected, and summarized information is shown in Table 1, both following the taxonomic classification reported by Hunt (2006).

**Results and Discussion**

*Reproductive isolation in Cactaceae.* Interspecific gene flow in Cactaceae, as in other angiosperms, is mediated by prezygotic barriers, which include asynchronous flowering periods, different pollination syndromes, and incompat-
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ible crosses, and postzygotic barriers, which prevent hybridization, avoiding the formation of seeds and reducing the quantity and quality of the progeny (Kay 2006, Baack et al. 2015). Very few studies have focused on the reproductive system of cacti, with less than 5% of the species having such evidence (Mandujano et al. 2010). Furthermore, there are few studies on the existence of barriers to interspecies genetic exchange (Mandujano et al. 2010, Montanucci 2015, Fachardo & Sigrist 2020), so knowledge about pollen-pistil interactions, as well as hybrid formation, is mainly based on inferences and extrapolations about what may be occurring in nature.

Pollination in Cactaceae is carried out mainly by bees, birds, bats and sphingid moths, as well as other insects, such as butterflies, beetles, wasps and ants, which have been reported to be relevant in some groups (Rosas & Pimiento 1986, Anderson 2001, Schlumberger 2011). Pollinators are responsible for transporting pollen from the androecium to the gynoecium, which accepts or rejects it through the compatibility system (Márquez Guzmán et al. 2013). In the case of this family, the pollination is mainly oligolectic, and there is evidence provided by experimental crosses that this system is not so selective. These crosses allow genetic exchange between individuals from different species, for example, *Opuntia* (Grant & Grant 1979, Griffith 2001), *Cylindropuntia* (Grant & Grant 1971), *Echinocereus* (Powell et al. 1991), and *Selenicereus* (Cisneros & Tel-Zur 2012), or even different genera such as *Pachycereus*, *Bergerocactus*, *Myrtilloccactus*, *Escontria*, *Cereus*, *Harrisia*, and *Consolea*, among others (Table S1, Rowley 1994, Anderson 2001). If this occurs, postzygotic barriers such as abortion of the hybrid embryo through the death of the endosperm occur, preventing seed formation (Marks 1966, Nishiyama & Yabuno 1979, Cisneros & Tel-Zur 2012). Another very important postzygotic barrier is the ploidy of the embryo because hybridization between diploid and tetraploid species can give rise to triploid zygotes, which have no stability in their chromosomal number and are aborted (Baack et al. 2015, Tel-Zur et al. 2020). Despite the aforementioned barriers, in Cactaceae, there is evidence of triploid hybrids in the genus *Cylindropuntia* (Pinkava 1999), while in *Selenicereus*, these hybrids do not survive or require a doubling of their chromosomes to survive (Tel-Zur et al. 2020).

Table 1. Summary of natural hybrids in Cactaceae following the taxonomic classification reported by Hunt et al. (2006). For more details on the genera and hybrids, see Table S1.

| Subfamily     | Tribe                  | No. of genera with hybrids | Hybridization type | No. of naturally occurring hybrids |
|---------------|------------------------|-----------------------------|--------------------|------------------------------------|
|               |                        |                             | Interspecific=a    | Intergeneric=b                     |                                    |
| Cactoideae    | Echinocereeae          | 8                           | a, b               | 9                                  |
|               | Hylocereeae            | 2                           | a                  | 6                                  |
|               | Cereae                 | 6                           | a, b               | 13                                 |
|               | Trichocereeae          | 14                          | a, b               | 16                                 |
|               | Cactae                 | 7                           | a, b               | 6                                  |
|               | Notocactae             | -                           | -                  | -                                  |
|               | Rhipsalidnear          | -                           | -                  | -                                  |
| Opuntioideae  | Cylindropuntiae        | 1                           | a                  | 11                                 |
|               | Opuntiae               | 3                           | a, b               | 38                                 |
|               | Austrocylindropuntiae  | -                           | -                  | -                                  |
|               | Pterocactae           | -                           | -                  | -                                  |
|               | Tephrocactae           | -                           | -                  | -                                  |
| Pereskioideae | -                      | -                           | -                  | -                                  |
| Maihuenioideae| -                      | -                           | -                  | -                                  |
Reproductive isolation in *Opuntia* has been proposed as low or nonexistent among certain members of the genus, mainly in North American species (Trujillo & González 1991, Majure et al. 2012b), while among South American species, there are fewer reports of hybridization, as well as fewer species (Hunt 2014). A study of two South American species of *Opuntia* demonstrated prezygotic reproductive isolation between *Opuntia elata* and *O. retrorsa*, which are sympatric in Brazil, while in North American species, there are reports of the absence of barriers to interspecific gene flow (Trujillo & González 1991, Griffith 2001), supporting the general idea that hybridization occurs less frequently among South American species (Anderson 2001, Hunt et al. 2006). Potential evidence of the importance of hybridization in the evolution of *Opuntia* can be found in the species richness, which is higher in North America than in South America; thus, where more hybridization events are present, there is higher species and subspecies diversity (Reyes-Agüero et al. 2006, Hernández et al. 2014, Hunt 2014).

The idea of hybrids in nature has attracted the attention of botanists who are often looking for individuals with intermediate characteristics; however, these types of individuals are often not found or do not exist. In other words, the hybrid might resemble one of the putative parental species, or it is not found in nature despite being viable when artificially obtained. For instance, this dilemma is present in *Astrophytum* due to common hybridization among cultivated species, but even when the species are sympatric in the wild, the corresponding hybrids are not seen in nature. Regarding this subject, in 2015, Montanucci investigated the reasons why there were no reports of natural hybrids between *Astrophytum coahuilense* and *A. capricorne* var. *senile*, although both are sympatric in northern Mexico and their flowering periods overlap. The author made experimental crosses, and in some cases, there was no fruit formation (prezygotic barriers), while in other cases, the germination of hybridized seeds was very low or led to the death of the seedling (postzygotic barriers). Therefore, not all sympatric species are able to hybridize, and not all hybrids have intermediate phenotypes with respect to their parental lineages because of the complexity of interactions such as dominance and epistasis, which makes phenotype prediction far from a simple average between two parents (Rosas et al. 2010).

As discussed earlier, little is known about all the processes that prevent or allow hybrid formation in Cactaceae, and it is necessary to develop more studies to improve our current understanding of the processes involved in hybridization and reproductive isolation of these species. Classic examples where this has been studied include *Iris* (Iridaceae) and *Helianthus* (Asteraceae), in which experimental crosses have been performed to better understand how the barriers to gene exchange act during hybrid formation, thus allowing crosses between certain species. In *Iris*, prezygotic barriers are decisive for hybrid formation, while in *Helianthus*, postzygotic barriers could reduce the expected number of hybrids (Anderson 1953, Heiser et al. 1969, Arnold 1994, Rieseberg 1995). Therefore, it is necessary to know in more detail the barriers to gene exchange in cacti, although a great challenge arises in columnar cacti of Echinocereae or Cereeae due to their long generation times (Anderson 2001). Studying pollination or performing experimental crosses in columnar cacti is quite complicated because flowers are usually found at the plant apex and reach a height of 12 m in *Cephalocereus* or 16 m in *Carnegiea* (Anderson 2001). Furthermore, generation times in columnar cacti range from 20 to 75 years; thus, performing experimental crosses, obtaining seeds and waiting until derived individuals grow and become sexually mature would be practically impossible (Copetti et al. 2017).

Relevance of hybrid speciation in angiosperms and Cactaceae. There are several processes by which speciation can occur. In some cases, speciation is mainly driven by selection and/or genetic drift, which often results in a pattern of fully resolved phylogenetic relationships (Gontier 2015). In contrast, unresolved phylogenetic relationships may be due to various factors, such as the lack of variation in molecular markers, incomplete lineage sorting, or hybridization (Soltis 2013). Reticulate evolution occurs when a lineage originates from the partial union of two different lineages (Linder et al. 2004). This process is characterized as being rapid and nongradual (Gontier 2015). Multiple examples of reticulated evolution in plants have been studied. One of the pioneers in this topic was Stebbins, who studied the genus *Crepis* (Asteraceae) by chromosome analysis, which allowed him to make the first proposals on the relevance of hybridization in evolution (Stebbins 1950). Later, in a joint work with Anderson, they concluded that hybridiza-
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tion plays a role in evolution because it produces new variation, which allows hybrids to occupy new niches (Anderson & Stebbins 1954). Therefore, it is relevant to analyze how reticulated evolution in other angiosperms has been studied and to compare those results with what has been observed in Cactaceae.

In Helianthus (Asteraceae) and Iris (Iridaceae), hybridization and hybrid speciation have been confirmed by experimental crosses and phylogenetic analysis, indicating a role for hybridization in the species diversity of these groups (Arnold 1997, 2006). When hybrid speciation is not accompanied by chromosome duplication, the process is known as homoploid speciation (Rieseberg 1991). On the other hand, hybridization involving chromosome doubling can also lead to speciation. This process is known as polyploid speciation (Rieseberg 1991), and since allopolyploid hybrids have extra genetic material in which mutations can occur, diversification of homologous genes is possible without losing the original function of the gene (Pinkava 2002, Glover et al. 2016).

An interesting example of polyploid speciation occurs in Fragaria (Rosaceae), a genus in which there are several allopolyploid species with a suspected hybrid origin. Through high-throughput sequencing and a specific methodology to analyze the origin of polyploids, which includes the assembly of haplotypes of low-copy nuclear genes, species trees and phylogenetic networks, the hybrid origin of polyploid species was demonstrated (Kamneva et al. 2017). In allopolyploid lineages, phylogenetic histories can be better represented using phylogenetic networks (Marcussen et al. 2011); thus, to understand reticulated evolution in polyploids, the use of phylogenetic networks allows a better understanding of the origin and relationships between species (Elworth et al. 2019). Most Fragaria polyploids have an allopolyploid origin; in other words, they have a hybrid origin (Kamneva et al. 2017), which can be extrapolated to other polyploid plant groups. Unfortunately, in Cactaceae, Opuntia and many other genera, it is not clear whether the plants are autopolyplids or allopolyploids, but because hybridization in Opuntia is so common, it is inferred that one of the main factors affecting polyploid formation is allopolyploidy combined with unreduced gametes (Majure et al. 2012a). Therefore, it is likely that a large number of polyploids in this genus are of hybrid origin.

Recently, the phylogenetic approach in conjunction with multiple lines of evidence has allowed us to verify complex processes such as hybridization. One remarkable use of multiple lines of evidence was performed in Campanula (Crowl et al. 2017), in which flow cytometry, morphometry and phylogenetic networks were used. Using all this evidence together, the authors found that an octoploid population of Campanula erinus is a hybrid whose parental species are the tetraploid cytotypes of C. erinus and C. creutzburgii. The use of several lines of evidence allows a better understanding of hybridization and its relevance in speciation events, so this kind of methodology should be used more often in Cactaceae, since all of the above methodologies have rarely been combined in Cactaceae. An example that uses multiple lines of evidence to test for hybridization between Escontria and Polaskia was performed by Cruz-Zamora and collaborators in 2017 (which is discussed in the section on the Cactoideae subfamily). Another genus in which several lines of evidence have been used together to corroborate artificial hybridization is Selenicereus (Cisneros & Tel-Zur 2010, Cisneros et al. 2013). Artificial hybridization promoted by humans in edible cacti likely impacted the artificial evolution of cultivated species in Selenicereus, Stenocereus, and Opuntia due to the selection of certain characteristics that are fixed and have resulted in multiple cultivars (Luna-Morales et al. 2001, Griffith 2004, Tel-Zur et al. 2020).

Hybridization coupled with polyploidy can give rise to new lineages and rapid speciation (Zhang et al. 2017, Meng et al. 2021). This process occurs in Rosaceae, a family known for hybridization and polyploidy in many of its genera, such as Cotoneaster, Fragaria, Malus, Micromeles, Rosa, Sorbus and other members of the Malinae subtribe (Meng et al. 2011, Kamneva et al. 2017, Hamston et al. 2018, Meng et al. 2021). Phylogenetic analysis using combined evidence from chloroplast (plastid markers or plastomes) and nuclear (low-copy genes) markers, morphology, and coalescent methods (species tree and/or phylogenetic networks) was performed to detect hybridization and to infer that this process is relevant for the diversification of this and other genera in Rosaceae (Meng et al. 2011, Kamneva et al. 2017, Zhang et al. 2017, Meng et al. 2021). Therefore, this kind of analysis should be performed in Cactaceae polyploids with evidence of hybridization, for example, Cylindropuntia, Echinocereus, Gymnocalycium, Opuntia, Pilosocereus, Selenicereus, and Weberbaueroerocereus, to reinforce the hypothesis of the relevance of hybrid
speciation and its impact on the diversification of these groups (Pinkava 2002, Hunt et al. 2006, Cisneros & Tel-Zur 2012, Majure et al. 2012b, Řepka & Mráček 2012, Guerrero et al. 2019).

Natural hybridization and its impact on Cactaceae evolution. Some studies have highlighted that evolution in Cactaceae involves processes such as polyploidy, hybridization and incomplete lineage sorting (ILS) (Machado 2008, Copetti et al. 2017, Guerrero et al. 2019), as well as morphological adaptations to extreme environments, emphasizing that the occurrence of these processes and their impact on evolution are quite complex (Pinkava 2002, Machado 2008, Majure et al. 2012b, Hernández-Hernández et al. 2014, Copetti et al. 2017). For example, chromosome numbers have been used to infer the ploidy of putative parental and hybrids, and given these results, it was found that the formation of polyploids has been of major importance in the speciation and adaptation of cytotypes to certain environmental conditions (Pinkava 2002, Majure et al. 2012a). Through a phylogenomic approach, incongruence between gene and species trees was found in Echinocereeae, probably due to long generation times and ILS, highlighting the importance of using multiple lines of evidence to understand phylogenetic relationships among morphologically similar species (Copetti et al. 2017). Cactaceae diversification involves multiple factors, such as hybridization, adaptations to dry environments, diverse pollination syndromes and growth forms related to water foraging and storage; therefore, environmental factors as well as those intrinsic to cacti species have resulted in the large number of species that exist in this family (Hernández-Hernández et al. 2014, Guerrero et al. 2019).

In Cactaceae, one of the main criteria to consider when determining if an individual plant is a hybrid is the presence of intermediate characteristics with respect to two morphologically differentiable species. A smaller proportion of hybrids have been reported based on chromosome comparisons, experimental crossings or the use of molecular markers (Anderson 2001, Hunt et al. 2006). The reports of natural hybrids in Cactaceae comprise two of the four recognized subfamilies, Cactoideae and Opuntioideae (Table 1). Hybrid formation in Cactoideae has been reported for six of the seven tribes, and this process occurs more frequently among species belonging to different genera than among members of the same genus (Table 1). On the other hand, in Opuntioideae, hybridization takes place more frequently between species from the same genera. The most diverse genus is Opuntia, which has highly variable morphological traits and is known for presenting multiple hybridization events, mainly due to weak barriers to genetic exchange and successful vegetative dispersion in hybrid lineages (Pinkava 2002, Hunt et al. 2006, Reyes-Agüero et al. 2006).

Cactoideae subfamily. In Cactoideae, natural hybridization may occur between diploid or polyploid species (Fig. 1A and B). It is important to highlight that although hybridization is reported between different genera (Table S1), which of course is possible, it could also be due to a deficient classification at the genus level or a classification that does not reflect the phylogenetic relationships of the analyzed species. Some examples of probable deficient classification include Carnegiea, Lophocereus, Pachycereus, and Stenocereus (Copetti et al. 2017) and the tribe Trichocereeae. A large number of reviewed studies have focused on reporting and testing hybridization using different approaches. In the literature, we found 54 articles about hybridization in Cactaceae, of which 36 analyzed Cactoideae species; the main approach used was morphological descriptions. The tribe with the highest number of natural hybrids is Trichocereeae, which is also one of the most diverse, and reports of hybrids were mainly inferred based only on morphological descriptions (Table S1). The phylogenetic relationships within this group have not been resolved, and the limits between genera and species based mainly on morphological characters are not clear (Guerrero et al. 2019). In this subfamily, it is necessary to reconstruct phylogenetic relationships and clarify generic boundaries because the highly variable morphological traits within the group might be providing an erroneous idea of hybridization when true relationships between genera remain unknown, as has happened in groups such as ferns (He & Zhang 2012).

Evolutionary implications of hybridization have been identified as important for Cactaceae, but very few hybridization cases have been tested. An interesting example occurs in hybrid zones of Melocactus in Brazil (Khan et al. 2020). This case assessed the impact of hybridization on parental genetic integrity but did not analyze the role of hybrids in population dynamics. The authors inferred reproductive barriers, but they did not conduct a formal test for this assertion. In Iris (Iridaceae), a gradual loss of reproductive isolation after the first hybridization event was
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reported (Arnold 2006). This loss could be occurring in Melocactus hybrid zones, where backcrosses are common, but because of natural selection and genetic drift, introgressed loci are being lost. Their population structure analyses showed F₁ hybrids as well as hybrids from subsequent backcrosses. Although the authors infer the existence of barriers to genetic exchange, the results indicate that these barriers are permeable. Thus, formal analyses of breeding systems must be performed to determine the dynamics of genetic exchange barriers in Melocactus as well as in species of Cactaceae. Finally, although hybridization was found in Melocactus, it seems that this process does not impact the genetic integrity or speciation of the studied populations.

Among columnar cacti, a recent study addressed hybridization between Polaskia chichipe and Escontria chiotilla (Cruz-Zamora et al. 2017), both inhabiting the Tehuacán-Cuicatlán biosphere reserve and sharing pollinators. Using morphological and genetic evidence, these species were identified as parents of hybrid individuals. It is relevant to mention that the authors also performed artificial crosses between parentals, whose hybrids were similar to the natural ones. This result demonstrates that despite the slow development in most Cactaceae members, it is possible to carry out experimental crosses to obtain hybridized progeny, as has been done in other plant groups, such as Helianthus (Asteraceae) (Rieseberg 1991). Therefore, studies of experimental crosses should be carried out between the putative parents of hybrids to validate putative hybrids observed in the wild.

In Cactaceae, there are many species with small populations that are threatened (Goettsch et al. 2015). Hence, the integrity of endemic or rare species is of great relevance for their conservation. One example is Sclerocactus, whose members inhabit western North America. In this genus, there is evidence of hybridization between Sclerocactus wetlandicus and S. brevispinus (Tepedino et al. 2010) and between S. glaucus and S. parviflorus (Schwabe et al. 2015). Hybridization can be an issue when populations are small due to interspecific gene flow, which might facilitate the fixation of introgressed alleles in species with smaller populations. One such case was studied in S. glaucus, which is endemic to western Colorado (United States of America). Introgression from S. parviflorus, a species with a wider distribution, was found in S. glaucus. However, the genetic integrity of S. glaucus seems to be intact; thus, the real threat to this species is anthropogenic pressures (Goettsch et al. 2015, Schwabe et al. 2015). It is also interesting that most Sclerocactus species are diploids (Rice et al. 2015); therefore, it would be relevant to know the ploidy of hybrids ploidy; if they have the same ploidy as their parents, homoploid hybridization would occur. This type of hybridization has been poorly documented in Cactoideae, and its study would be worth addressing to better understand speciation within this subfamily (Rieseberg 1991, Arnold 1997).

Although most of the articles focus on Cactoideae, there are still many genera for which there are no reports of natural hybridization despite evidence of hybridization in cultivation. One interesting case is Mammillaria. It is important to note that this is the genus with the highest richness in the subfamily, for which little is known about natural hybridization (Hunt 1977); however, hybridization in cultivation is common. For example, hybridization in cultivation is possible between Turbinicarpus and Thelocactus, as well as in cultivated species from Epiphyllum, Selenicereus and Aporocactus, among other species (Cullmann et al. 1987). Although these reports do not constitute formal scientific studies, they allow us to better understand the presence or absence of barriers to gene exchange.

We can conclude that the reports of hybrids in the Cactoideae subfamily are mainly based on the description of strange individuals with intermediate morphological traits. Previous statements suggest an important role of hybridization in the evolution of Cactoideae, but this should be better supported by performing formal analyses as well as integrating multiple sources of evidence. Thus, it is necessary to perform phylogenetic and reticulation analyses as well as experimental crosses to formally test hybridization hypotheses and determine the impact of this process on the evolution of Cactoideae.

Opuntioideae subfamily. Although Opuntioideae is known as a group with frequent natural and artificial hybridization, we only found 18 articles on this subject. This result could be related to the presence of deterrent traits, such as spines and glochids, that hinder their collection. During the 1990s and the first decade of the 21st century, natural hybridization within Opuntioideae in North America was studied by Pinkava and collaborators, whose compilation of studies in Opuntia and Cylindropuntia contributed to the understanding of the evolutionary implications of
this process in the subfamily (Baker & Pinkava 1987, Pinkava 1999). Their studies include data from taxonomic treatments, chromosomal counts, and morphometric and biogeographic analysis, which led to the postulation that the members of this subfamily tend to have rapid speciation due to their often small and isolated populations, their perennial habit, vegetative multiplication (Bobich & Nobel 2001, Figure 1), apomixis and allopolyploidization, thus allowing the accumulation of heritable mutations in their descendants. Vegetative reproduction impacts evolutionary processes because it helps maintain hybrid genotypes, which can later reproduce sexually with parental genotypes or other species (Arnold 2006). The same pattern has been reported in Opuntia due to high levels of clonal reproduction and the presence of putative hybrids (Pinkava 2002, Reyes-Agüero et al. 2006). A prominent example using the abovementioned evidence can be found in the Opuntia polyacantha complex, within which the authors found differences in chromosome numbers at the boundaries of the species distribution, allowing them to infer the process of peripatric speciation (Mayr 1954, Pinkava 2002).

In Opuntioideae hybridization events have been documented using chromosome numbers. For example, within Opuntia populations, there is variation in chromosome numbers that is sometimes associated with hybridization events and subsequent chromosome duplication, which occurs to ensure the correct pairing of chromosomes, thus allowing the subsequent reproduction of the hybrids (Soltis et al. 2003, Tel-Zur et al. 2020). Chromosome doubling does not always occur, and putative hybrids may have originated by the union of unreduced gametes (Pinkava 2002). To classify species complexes in Opuntia, it is important to understand the ploidy of species throughout their distribution and therefore to know which species are related, if they form hybrids and the boundaries between species. One such example is the Opuntia humifusa complex (Majure et al. 2017). Another remarkable example of the use of chromosome counts is in Opuntia s. l. from South America; notably, ploidy numbers ranged from diploid (2x) to pentaploid (5x), and important cytological characteristics to understand hybridization patterns such as chromosomal terminal satellites were found in Opuntia aurantiaca (3x) and O. salmiana (3x) (Realini et al. 2014).

Interestingly, because of the slow growth of most cacti, few studies have evaluated breeding systems and tested the hybridization hypothesis using reciprocal crosses. However, experiments performed in prickly pear from the Chihuahuan Desert found five combinations of interfertile taxa involving seven species and four varieties (Griffith 2001), and experimental crosses in northern Mexico between Opuntia streptacantha, O. robusta, O. leucotricha, and O. rastrera showed that all studied species were interfertile (Trujillo & González 1991). These kinds of experiments are important to support putative natural hybridization events, and documentation of intermediate morphological traits, ploidy levels, and shared biparentally inherited markers are conclusive evidence for hybridization cases in North American Opuntia (Griffith 2003). Species in this part of the continent are more abundant, and more hybridization cases have been reported, with this genetic exchange as well as polyploidy likely causing a higher speciation rate (Soltis & Soltis 2009, Soltis 2013).

Another widely studied genus is Cylindropuntia, in which 10 of the 33 species recognized by Hunt et al. (2006) form natural hybrids, most of which are triploids (3x) with vegetative multiplication. These triploid individuals were useful for better understanding the genetic exchange between species with different ploidies. Through these analyses, it was shown that the formation of triploids was generally via nonreduced gametes and not because of crosses between diploid (2x) and tetraploid (4x) individuals (Baker & Pinkava 1987, Pinkava 2002). Other authors obtained information about hybrid fertility, showing that triploid individuals were not fully infertile (Grant & Grant 1971). Through these studies, hybrid formation was inferred in Cylindropuntia, and it was demonstrated that hybrids with odd ploidy are still capable of breeding; thus, backcrosses may occur, giving rise to new lineages (Arnold 1997).

Characterizing the ploidy in Opuntioideae and other Cactaceae species is of crucial importance because it allows us to understand their speciation patterns. In Cactoideae, homoploid hybridization could frequently occur undetected because most of the species are diploid (Baker et al. 2009, Rice et al. 2015, Baker & Pinkava 2018), whereas in polyploid Opuntioideae, allopolyploid speciation has been detected through chromosome counts and molecular markers (Majure et al. 2012a,b). Despite its relevance, the study of ploidy levels is less used in Cactoideae, whereas in Opuntioideae, it is still relevant due to the large number of polyploids and their reticulate evolution (Soltis & Soltis 2009, Majure et al. 2012a). It has been proposed that polyploidy is a condition that allows rapid speciation and evolution...
in plants and is frequent in Opuntioideae (Stebbins 1971, Hunt 2014). The origin of polyploidy in plants may be due to somatic duplication in meristems, unreduced gametes or hybridization, with the last two being the most frequent in Opuntioideae (Otto & Whitton, 2000, Pinkava 2002, Majure et al. 2012a,b).

Different from what might be expected, not all species in Opuntioideae present hybridization. We searched specifically for papers on hybridization in Andean species from the genera Maihueniopsis, Austrocylindropuntia and Cumulopuntia, but there were no reports or molecular evidence of hybridization (Anderson 2001, Ritz et al. 2012). Phylogenetic analysis performed on Tephrocacteae as well as the previously mentioned genera using plastid and nuclear markers yielded almost the same trees, so no evidence of hybridization was found (Ritz et al. 2012). In Tephrocactus, no discordant phylogenetic histories were found, although in previous karyotypic analysis, evidence of allopolyploidy was found for *T. recurvatus* (Las Peñas et al. 2009, Ritz et al. 2012), so more studies should be conducted using multiple sources of evidence to understand the evolutionary history of South American species.

Finally, although there is more formal evidence for hybridization in Opuntioideae, more analysis focused on reticulated evolution should be performed on poorly studied species to better understand the impact of hybridization on their speciation processes, as well as to know why hybridization did not occur in certain lineages when hybridization in their sister lineages is very common. We can infer that the process of hybrid speciation plays an important role in the most commonly hybridizing genera *Opuntia* and *Cylindropuntia* because they are also the most diverse in the Opuntioideae, although more comprehensive analysis is still needed to formally test this hypothesis.

*Discordant phylogenetic stories in Opuntioideae.* Through phylogenetic analysis, we can understand the ancestor-descendant relationship for focal taxa, and although it is not their main purpose, some patterns on phylogenetic trees have been used to infer hybridization. One of the most commonly used patterns is that between phylogenetic trees obtained from molecular markers of uniparental versus biparental inheritance (Arnold 1997, Sang et al. 1997, Russell et al. 2010, Meng et al. 2011).

The phylogenetic history of Opuntioideae is complicated, and although it has been studied by different groups of botanists over time, its phylogenetic relationships are not yet fully understood. Traditional species recognition involves morphological characters, but in Opuntioideae, the high variability of morphological traits within species makes the use of molecular, cytological, biogeographic and reproductive evidence necessary to classify species from this subfamily (Pinkava 2002, Majure et al. 2014, 2017).

Phylogenetic analysis has been performed on most members of the subfamily, mainly using chloroplast markers because the use of nuclear markers has led to discordant nodes in some cases. An initial approach to detecting hybridization is performing phylogenetic analyses using molecular markers with uniparental inheritance (chloroplast) compared to biparental inheritance (nuclear). If there is incongruence between phylogenetic trees, one likely cause is hybridization, although ILS is also a possibility (Arnold 1997, Elworth et al. 2019). One interesting example of phylogenetic incongruence involves the genus *Consolea*, which was suggested to have a hybrid origin due to its morphologically intermediate characters when compared with *Brasilioripuntia*, *Opuntia*, and *Tacinga*, and because it has not been possible to know with certainty its phylogenetic position. Initially, this genus was recovered outside *Opuntia sensu stricto* (s.s.) (Wallace & Dickie 2002). Subsequently, *Consolea* was found inside *Opuntia* s.s. (Griffith & Porter 2009), and finally, in a combined analysis (with plastid and nuclear markers), *Consolea* was found outside *Opuntia* s.s., but the analysis was performed including only diploid species (Majure et al. 2012b). Most *Consolea* species are polyploids (Negrón-Ortiz 2007), and their autoploid or allopolyploid origin should be studied with approaches such as high-throughput sequencing.

Another remarkable case involves *Nopalea*, which was previously considered outside *Opuntia* due to its flowers and pollination syndrome, but phylogenetically, it is nested within *Opuntia* s.s.; therefore, it is now considered part of this genus, providing a remarkable example of morphological characteristics in Opuntioideae leading to an artificial classification. Additionally, there is evidence that members from this clade hybridize with members of the clades Basilaes, Scheeriane and *Opuntia* s. l. In *Opuntia*, it has been observed that hybrids survive only if the pollen donor has higher ploidy than the female receptor (Griffith 2001). Most species previously known as *Nopalea* have diploid
chromosome numbers, so they may act as pollen receptors for species with higher chromosome numbers, explaining the large number of hybridization events with species from other clades.

Phylogenetic analyses in *Opuntia* are complicated due to widespread polyploidy. For species in this genus, polyploidy represents an important evolutionary adaptation for survival. For example, it has been found that in some northern polyploid lineages, this condition allows them to better adapt to low temperatures up to -48 °C (Nobel & Bobich 2002). For example, polyploid lineages of *Opuntia humifusa* and *O. macrorhiza* can tolerate very cold temperatures, and their diploid lineages inhabit places with warmer temperatures (Majure *et al.* 2017). Although polyploidy is relevant for the survivorship of *Opuntia* species, it is also a condition that makes the interpretation of the evolutionary relationships between species difficult. A clear example is presented in the most complete phylogenetic analysis for the genus, in which it is possible to define large groups within *Opuntia* s.s. when excluding hybrid and polyploid taxa. More than 50 % of *Opuntia* species are polyploids, and this condition can occur due to the union of unreduced gametes and/or hybridization (Pinkava 2002). Having this estimated and extremely high number of polyploids could suggest a considerable number of diversification events likely of hybrid origin, either between populations of the same species or of different species. In addition, it is important to emphasize that it is necessary to carry out phylogenetic analyses using other kinds of molecular information, such as genomics, transcriptomics, proteomics, plastomics or nuclear targeted regions, and including these polyploid species to determine their relationship with the remaining diploid species.

**Conclusions**

We conclude that in Cactaceae, it is necessary to improve and accelerate the formal study of isolation barriers between noncultivated species from subfamilies with reports of hybrids. More efforts should be made to analyze hybridization in Opuntioideae because, compared to Cactoideae, there are fewer articles available. From 2008 to the article search in 2021, only seven articles have been published on this topic for Opuntioideae, and in Cactoideae, the effort should include integrating formal evidence to test for hybridization. In particular, hybridization reports in tribe Trichocereeae should be revised because of the complex relationships among their members, which can lead to the inference of false hybridization events between different genera. For Opuntioideae, it is a priority to know the chromosome numbers across all species, their phylogenetic relationships, and the boundaries between species, which have been difficult to elucidate due to hybridization and polyploidy. The analysis of a process as complex as hybridization must be comprehensive and should include ecological, reproductive, morphological, molecular, and evolutionary evidence to better understand the dynamics of hybrids, such as their primary formation, their demise or their establishment as a new species. Independently studied processes, such as pollination and the interaction between pollen and pistil, polyploid chromosome arrangements, how chromosomes and genes segregate in a newly formed hybrid, the establishment of new hybrid populations, and their separation from parental species, must be studied in an integrative context. In the case of reproductive biology, it is necessary to carry out studies in those species whose pollen–pistil interactions are unknown. For cytogenetics, it is necessary to know the chromosomal numbers of studied species, as well as the different cytotypes that coexist within a species. From the phylogenetic and population genetics perspective, it is necessary to perform analyses that include a greater number of individuals, variable markers, coalescence analysis and phylogenetic networks to understand the complex processes that occur when two distinct lineages are mixed. Taxonomic studies also need to consider the possibility that species may be flexible and that their identity can be modified by processes such as hybridization. Taxonomic studies also need to be used to understand the limits to distinguishing species despite the existence of hybrid individuals, including the use of all possible evidence and formal morphological analysis with statistical analysis, thus departing from species being described by using only the taxonomist criterion and moving towards describing Cactaceae species as a whole and not based on just one individual. All of the abovementioned recommendations will improve the understanding of hybridization and evolution processes in the interesting and beautiful group of plants popularly known as cacti.
Supplementary material

Supplemental data for this article can be accessed here: https://doi.org/10.17129/botsci.3065

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Literature cited

Anderson E. 1953. Introgressive hybridization. Biological Reviews 28: 280-307. DOI: https://doi.org/10.1111/j.1469-185X.1953.tb01379.x
Anderson EF. 2001. The Cactus family. Portland, Oregon: Timber Press. ISBN: 9780881924985
Anderson E, Stebbins GL. 1954. Hybridization as an evolutionary stimulus. Evolution 8: 378-388. DOI: https://doi.org/10.2307/2405784
Arias S, Gama S, Vázquez B, Guzmán LU. 2012. Flora del Valle de Tehuacán-Cuicatlán. Fascículo 95, Cactaceae. México: Instituto de Biología, Universidad Nacional Autónoma de México 95. ISBN 9786070230790
Arnold ML. 1994. Natural hybridization and Louisiana Irises. BioScience 44: 141-147. DOI: https://doi.org/10.1105/tpc.160370
Arnold ML. 1997. Natural hybridization and evolution. Nueva York: Oxford University Press. ISBN: 9780195356687
Arnold ML. 2004. Transfer and origin of adaptations through natural hybridization: were Anderson and stebbins right? The Plant Cell 16: 562-570. DOI: https://doi.org/10.1105/tpc.160370
Arnold ML. 2006. Evolution through genetic exchange. Oxford University Press ISBN: 9780199229031
Baack E, Melo MC, Rieseberg LH, Ortiz-Barrientos D. 2015. The origins of reproductive isolation in plants. New Phytologist 207: 968-984. DOI: https://doi.org/10.1111/nph.13424
Baker MA, Pinkava DJ 1987. A cytological and morphometric analysis of a triploid apomict, Opuntia × kelvinensis (subgenus Cylindropuntia, Cactaceae). Brittonia 39: 387-401. DOI: https://doi.org/10.2307/2807141
Baker MA, Pinkava, DJ. 2018. Chromosome numbers in some cacti of western North America - IX. Haseltonia 25: 2-29. DOI: https://doi.org/10.2985/026.025.0103
Baker MA, Rebman JP, Parfitt BD, Pinkava DJ, Zimmerman AD. 2009. Chromosome numbers in some cacti of western North America - VIII. Haseltonia, 15: 117-134. DOI: https://doi.org/10.2985/026.015.0112
Bobich EG, Nobel PS. 2001. Biomechanic and anatomy of cladode junctions for two Opuntia (Cactaceae) species and their hybrid. American Journal of Botany 88: 391-400. DOI: https://doi.org/10.2307/2657103
Britton NL, Rose JN. 1919. The Cactaceae: descriptions and illustrations of plants of the cactus family. Washington: Carnegie Institution of Washington. ISBN: 9780598344809
Britton NL, Rose JN. 1920. The Cactaceae: descriptions and illustrations of plants of the cactus family. Washington: Carnegie Institution of Washington. ISBN: 9780598344830
Britton NL, Rose JN. 1922. The Cactaceae: descriptions and illustrations of plants of the cactus family. Washington: Carnegie Institution of Washington. ISBN: 9781236575883
Britton NL, Rose JN. 1923. The Cactaceae: descriptions and illustrations of plants of the cactus family. Washington: Carnegie Institution of Washington. ISBN: 9781276473439
Butterworth CA, Wallace RS. 2005. Molecular phylogenetics of the leafy cactus genus Pereskia (Cactaceae). Systematic Botany 30: 800-808.
Cisneros A, Benega R, Tel Zur N. 2013. Creation of novel interspecific-interploid Hylocereus hybrids (Cactaceae) via embryo rescue. Euphytica 189. DOI: https://doi.org/10.1007/s10681-012-0800-3

Cisneros A, Tel-Zur N. 2010. Embryo rescue and plant regeneration following interspecific crosses in the genus Hylocereus (Cactaceae). Euphytica 174: 73-82. DOI: https://doi.org/10.1007/s10681-010-0135-x

Cisneros A, Tel-Zur N. 2012. Evaluation of interspecific-interploid hybrids (F1) and back crosses (BC1) in Hylocereus species (Cactaceae). In: Swan A. ed. Meiosis. Rijeka: IntechOpen, pp.20. DOI: https://doi.org/10.5772/32435

Critchfield WB. 1986. Hybridization and classification of the white pines (Pinus section Strobus). Taxon 35: 647-656. DOI: https://doi.org/10.2307/1221606

Crowl AA, Myers C, Cellinese N. 2017. Embracing discordance: Phylogenomic analyses provide evidence for allopolyploidy leading to cryptic diversity in a Mediterranean Campanula (Campanulaceae) clade. Evolution; International Journal of Organic Evolution 71: 913-922. DOI: https://doi.org/10.1111/evo.13203

Cullmann W, Goetz E, Gröner G. 1987. The encyclopedia of cacti. Timber Press.

Elworth RAL, Ogilvie HA, Zhu J, Nakhele L. 2019. Advances in computational methods for phylogenetic networks in the presence of hybridization. In: Warnow T. ed. Bioinformatics and phylogenetics: seminal contributions of Bernard Moret. Cham: Springer International Publishing, pp. 317-360. ISBN: 9783030108373

Fachardo ALS, Sigrist MR. 2020. Pre-zygotic reproductive isolation between two synchronopatric Opuntia (Cactaceae) species in the Brazilian Chaco. Plant Biology 22: 487-493. DOI: https://doi.org/10.1111/plb.13077

Glover NM, Redestig H, Dessimoz C. 2016. Homoeologs: What are they and how do we infer them? Trends in Plant Science 21: 609-621. DOI: https://doi.org/10.1016/j.tplants.2016.02.005

Goettensch B, Hilton-Taylor C, Cruz-Piñón G, Duffy JP, Frances A, Hernández HM, Inger R, Pollock C, Schipper J, Superina M, Taylor NP, Tognelli M, Abba AM, Arias S, Arreola-Nava HJ, Baker MA, Bárdenas RT, Barrios D, Gaston KJ. 2015. High proportion of cactus species threatened with extinction. Nature Plants 1: 1-7. DOI: https://doi.org/10.1038/nplants.2015.142

Gontier N. 2015. Reticulate evolution everywhere. In: Gontier N. ed. Reticulate evolution: symbiogenesis, lateral gene transfer, hybridization and infectious heredity. Springer International Publishing, pp. 1-40. https://doi.org/10.1007/978-3-319-16345-1_1

Granados-Aguilar X, Granados Mendoza C, Cervantes CR, Montes JR, Arias S. 2021. Unraveling reticulate evolution in Opuntia (Cactaceae) from Southern Mexico. Frontiers in Plant Science 11: 606809. DOI: https://doi.org/10.3389/fpls.2020.606809

Grant V, Grant KA. 1971. Natural hybridization between the cholla cactus species Opuntia spinosior and Opuntia versicolor. PNAS 68: 1993-1995. DOI: https://doi.org/10.1073/pnas.68.9.1993

Grant V, Grant KA. 1979. Hybridization and Variation in the Opuntia phaeacantha group in Central Texas. Botanical Gazette 140: 208-215. DOI: https://www.jstor.org/stable/2473720

Griffith PM. 2001. Experimental hybridization of northern Chihuahuan desert region Opuntia (Cactaceae). A Journal of Systematic and Evolutionary Botany 20: 37-42. DOI: Griffith PM. 2003. Using molecular evidence to elucidate reticulate evolution in Opuntia (Cactaceae). Madroño 50: 162-169.

Griffith PM. 2004. The origins of an important cactus crop, Opuntia ficus-indica (Cactaceae): new molecular evidence. American Journal of Botany 91: 1915-1921. DOI: https://doi.org/10.3732/ajb.91.11.1915

Griffith PM, Porter M. 2009. Phylogeny of Opuntioidae (Cactaceae). International Journal of Plant Sciences 170: 107-116. DOI: https://www.jstor.org/stable/10.1086/593048
Hybridization processes in Cactaceae

Guerrero PC, Majure LC, Cornejo-Romero A, Hernández-Hernández T. 2019. Phylogenetic relationships and evolutionary trends in the cactus family. *Journal of Heredity* **110**: 4-21. DOI: [https://doi.org/10.1093/jhered/esy064](https://doi.org/10.1093/jhered/esy064)

Márquez Guzmán J, Collazo Ortega M, Martínez Gordillo M, Orozco Segovia A, Vázquez Santana S 2013. *Biología de angiospermas*. UNAM, Facultad de Ciencias. ISBN: 97 86070227059

Hamston TJ, de Vere N, King RA, Pellicer J, Fay MF, Cresswell JE, Stevens JR. 2018. Apomixis and hybridization drives reticulate evolution and phylectic differentiation in *Sorbus* L.: Implications for conservation. *Frontiers in Plant Science* **9**. DOI: [https://doi.org/10.3389/fpls.2018.01796](https://doi.org/10.3389/fpls.2018.01796)

He LJ, Zhang XC. 2012. Exploring generic delimitation within the fern family Thelypteridaceae. *Molecular Phylogenetics and Evolution* **65**: 757-764. DOI: [https://doi.org/10.1016/j.ympev.2012.07.021](https://doi.org/10.1016/j.ympev.2012.07.021)

Heiser CB, Smith DM, Clevenger SB, Martin WC. 1969. The North American sunflowers (*Helianthus*). *Memoirs of the Torrey Botanical Club* **22**: 1-218.

Hernández H, Gómez-Hinostrosa C, Bárcenas R, Puente-Martinez R, Reyes-Agüero J. 2014. A checklist of the subfamily Opuntioideae (Cactaceae) in North and Central America. *In: Hunt D. ed. Further studies in the Opuntioideae (Cactaceae).* England: DH Books, pp. 185-200. ISBN: 9780953813414

Hernández-Hernández T, Brown JW, Schlumberger BO, Eguiarte LE, Magallón S. 2014. Beyond aridification: multiple explanations for the elevated diversification of cacti in the New World Succulent Biome. *New Phytologist* **202**: 1382-1397. DOI: [https://doi.org/10.1111/nph.12752](https://doi.org/10.1111/nph.12752)

Hunt DR. 1977. The classification of *Mammillaria*. *The National Cactus and Succulent Journal* **32**: 75-81.

Hunt DR. 2014. An interim checklist of the subfamily Opuntioideae (Cactaceae) in the Caribbean region and South America. *In: Hunt D. ed. Further studies in the Opuntioideae (Cactaceae).* England: DH Books, pp. 201-223. ISBN: 9780953813414

Hunt DR, Taylor NP, Charles G. 2006. *The new cactus lexicon*: Text. Inglaterra: DH books. ISBN: 9780953813452.

Jensen R J, Hokanson SC, Isebrands JG, Hancock JF. 1993. Morphometric variation in oaks of the apostle islands in Wisconsin: evidence of hybridization between *Quercus rubra* and *Q. ellipsoidalis* (Fagaceae). *American Journal of Botany* **80**: 1358-1366. DOI: [https://doi.org/10.2307/2445721](https://doi.org/10.2307/2445721)

Kamneva OK, Syring J, Liston A, Rosenberg NA. 2017. Evaluating allopolyploid origins in strawberries (*Fragaria*) using haplotypes generated from target capture sequencing. *BMC Evolutionary Biology* **17**: 180. DOI: [https://doi.org/10.1186/s12862-017-1019-7](https://doi.org/10.1186/s12862-017-1019-7)

Kay KM. 2006. Reproductive isolation between two closely related hummingbird pollinated neotropical gingers. *Evolution* **60**: 538-552. DOI: [https://doi.org/10.1111/j.0014-3820.2006.tb01135.x](https://doi.org/10.1111/j.0014-3820.2006.tb01135.x)

Khan G, Franco FF, Silva GAR, Bombonato JR, Machado M, Alonso DP, Ribolla PEM, Albach DC, Moraes EM. 2020. Maintaining genetic integrity with high promiscuity: Frequent hybridization with low introgression in multiple hybrid zones of *Melocactus* (Cactaceae). *Molecular Phylogenetics and Evolution* **142**: 106642. DOI: [https://doi.org/10.1016/j.ympev.2019.106642](https://doi.org/10.1016/j.ympev.2019.106642)

Korotkova N, Aquino D, Arias S, Eggli U, Franck A, Gómez-Hinostrosa C, Guerrero PC, Hernández HM, Kohlbecker A, Köhler M, Luther K, Majure LC, Müller A, Metzing D, Nyffeler R, Sánchez D, Schlumberger B, Berendsohn WG. 2021. Cactaceae at Caryophyllales.org - a dynamic online species-level taxonomic backbone for the family. *Willdenowia* **51**: 251-270. DOI: [https://doi.org/10.3372/wi.51.51208](https://doi.org/10.3372/wi.51.51208)

Korotkova N, Borsch T, Arias S. 2017. A phylogenetic framework for the Hylocereae (Cactaceae) and implications for the circumscription of the genera. *Phytotaxa* **327**: 1-46. DOI: [https://doi.org/10.11646/phytotaxa.327.1.1](https://doi.org/10.11646/phytotaxa.327.1.1)

Las Peñas ML, Urdampilleta JD, Bernardello G, Forni-Martins ER. 2009. Karyotypes, heterochromatin, and physical mapping of 18S-26S rDNA in Cactaceae. *Cytogenetic and Genome Research* **124**: 72-80. DOI: [https://doi.org/10.1159/000200090](https://doi.org/10.1159/000200090)

Linder C, Moret B, Nakhle L, Warnow T. 2004. Network (reticulate) evolution: biology, models, and algorithms. *The Pacific Symposium on Biocomputing*. [https://psb.stanford.edu/previous/psb04/tut_linder.html](https://psb.stanford.edu/previous/psb04/tut_linder.html) (accessed October 18, 2021).
Granados-Aguilar et al. / Botanical Sciences 100 (4): 797-813. 2022

Luna-Morales CDC, Rivera JRA, Peña-Valdivia CB. 2001. Cultivares tradicionales mixtecos de *Stenocereus pruinatus* y *S. stellatus* (Cactaceae) *Anales del Instituto de Biología. Serie Botánica* 72: 131-155.

Machado MC. 2008. What is the role of hybridization in the evolution of the Cactaceae? *Bradleya* 26: 1-18. DOI: https://doi.org/10.25223/brad.n26.2008.a1

Majure LC, Judd WS, Soltis PS, Soltis DE. 2012a. Cytogeography of the Humifusa clade of *Opuntia s.s.* Mill. 1754 (Cactaceae, Opuntioideae, Opuntieae): correlations with Pleistocene refugia and morphological traits in a polyploid complex. *Comparative Cytogenetics* 6: 53-77. DOI: https://doi.org/10.3897/CompCytogen.v6i1.2523

Majure LC, Judd WS, Soltis PS, Soltis DE. 2017. Taxonomic revision of the *Opuntia humifusa* complex (Opuntieae: Cactaceae) of the eastern United States. *Phytotaxa* 290: 1-65. DOI: https://doi.org/10.11646/phytotaxa.290.1.1

Majure LC, Puente R, Griffith MP, Judd WS, Soltis PS, Soltis DE. 2012b. Phylogeny of *Opuntia s.s.* (Cactaceae): Clade delineation, geographic origins, and reticulate evolution. *American Journal of Botany* 99: 847-864. DOI: https://doi.org/10.3732/ajb.1100375

Majure LC, Soltis DE, Soltis PS, Judd WS. 2014. A case of mistaken identity, *Opuntia abjecta*, long-lost in synonymy under the Caribbean species, *O. triacantha*, and a reassessment of the enigmatic *O. cubensis*. *Brittonia* 66: 118-130. DOI: https://doi.org/10.1007/s12228-013-9307-z

Mandujano MC, Carrillo-Angeles I, Martínez-Peralta C, Golubov J. 2010. Reproductive biology of Cactaceae. In: Ramawat KG. ed. *Desert plants: Biology and biotechnology*. Berlin: Springer, 197-230. DOI: https://doi.org/10.1007/978-3-642-02550-1_10

Marcussen T, Jakobsen KS, Danihelka J, Ballard HE, Blaxland K, Brysting AK, Oxelman B. 2011. Inferring species networks from gene trees in high-polyploid north American and Hawaiian violets (*Viola*, Violaceae). *Systematic Biology* 61: 107-126. DOI: https://doi.org/10.1093/sysbio/syr096

Masks GE. 1966. The origin and significance of intraspecific polyploidy: Experimental evidence from *Solanum chacoense*. *Evolution* 20: 552-557. DOI: https://doi.org/10.2307/2406589

Mayr E. 1954. Change of genetic environment and evolution. In: Huxley J, Hardy AC. eds. *Evolution as a Process* (London: Unwin Brothers), pp. 157-180. ISBN: 0045750092

Meng J, Fougere-Danezan M, Zhang LB, Li DZ, and Yi TS. 2011. Untangling the hybrid origin of the Chinese tea roses: Evidence from DNA sequences of single-copy nuclear and chloroplast genes. *Plant Systematic and Evolution* 297: 157-170. DOI: https://doi.org/10.1007/s00606-011-0504-5

Meng KK, Chen SF, Xu KW, Zhou RC, Li MW, Dhamala MK, Liao WB, Fan Q. 2021. Phylogenomic analyses based on genome-skimming data reveal cyto-nuclear discordance in the evolutionary history of *Cotoneaster* (Rosaceae). *Molecular Phylogenetics and Evolution* 158: 107083. DOI: https://doi.org/10.1016/j.mpev.2021.107083

Montanucci RR. 2015. Experimental evidence for reproductive isolation between *Astrophytum coahuilense* (H. Möller) Kayser and *A. Capricorne* var. *senile* (Fric) Okumura (Cactaceae). *Haseltonia* 20: 13-21. DOI: https://doi.org/10.2985/026.020.0104

Morales-Briones DF, Romoleroux K, Kolář F, Tank DC. 2018. Phylogeny and evolution of the neotropical radiation of *Lachemilla* (Rosaceae): Uncovering a history of reticulate evolution and implications for infrageneric classification. *Systematic Botany* 43: 17-34. DOI: https://doi.org/10.1600/036364418X696897

Negrón-Ortiz V. 2007. Chromosome numbers, nuclear DNA content, and polyploidy in *Consolea* (Cactaceae), an endemic cactus of the Caribbean Islands. *American Journal of Botany* 94: 1360-1370. DOI: https://doi.org/10.3732/ajb.94.8.1360

Nishiyama I, Yabuno T. 2007. Triple fusion of the primary endosperm nucleus as a cause of interspecific cross-incompatibility in *Avena*. *Euphytica* 28: 57-65. DOI: https://doi.org/10.1007/BF00029173

Noblet PS & Bobich EG. 2002. Environmental biology. In: Noblet PS ed. *Cacti: biology and uses*. Berkeley, CA: University of California Press, pp 57-74. ISBN: 9780520297889

Otto SP, Whitton J. 2000. Polyploid incidence and evolution. *Annual Review of Genetics* 34: 401-437. DOI: https://doi.org/10.1146/annurev.genet.34.1.401
Hybridization processes in Cactaceae

Pinkava DJ. 1999. Cactaceae Cactus family: Part three: *Cylindropuntia* (Engelm.) Knuth Chollas. *Journal of the Arizona-Nevada Academy of Science* **32**: 32-47.

Pinkava DJ. 2002. On the evolution of the North American Opuntioideae. In: Hunt D. ed. *Studies in the Opuntioideae (Cactaceae).* England: DH Books, pp. 59-98. ISBN: 9780953813414

Powell AM, Zimmerman AD, Hilsenbeck RA. 1991. Experimental documentation of natural hybridization in Cactaceae: origin of Lloyd’s Hedgehog Cactus, *Echinocereus × lloydii.* *Plant Systematics and Evolution* **178**: 107-122.

Realini M, Gottlieb A, Font F, Picca P, Poggio L, Gonzalez G. 2014. Cytogenetic characterization of southern South American species of *Opuntia s. l.* (Cactaceae-Opuntioideae). In: Hunt D. ed. *Further Studies in the Opuntioideae.* England: DH Books, pp. 31-50. ISBN: 9780953813414

Řepka R, Mrácek, J. 2012. *Gymnocalycium esperanzae:* A nothospecies? *Haseltonia* **18**: 105-115. DOI: https://doi.org/10.2985/026.018.0113

Reyes-Agüero JA, Aguirre R, Valiente-Banuet A. 2006. Reproductive biology of *Opuntia:* A review. *Journal of Arid Environments* **64**: 549-585. DOI: https://doi.org/10.1016/j.jaridenv.2005.06.018

Rice A, Glick L, Abadi S, Einhorn M, Kopelman NM, Salman-Minkov A, Mayzel J, Chay O, Mayrose I. 2015. The chromosome counts database (CCDB) - a community resource of plant chromosome numbers. *New Phytologist* **206**: 19-26. DOI: https://doi.org/10.1111/nph.13191

Rieseberg LH. 1991. Homoploid reticulate evolution in *Helianthus* (Asteraceae): Evidence from ribosomal genes. *American Journal of Botany* **78**: 1218-1237. DOI: https://doi.org/10.2307/2444926

Rieseberg LH. 1995. The role of hybridization in evolution: Old wine in new skins. *American Journal of Botany* **82**: 944-953. DOI: https://doi.org/10.2307/2445981

Ritz CM, Reiker J, Charles G, Hoxey P, Hunt D, Lowry M, Stuppy W, Taylor N. 2012. Molecular phylogeny and character evolution in terete-stemmed Andean opuntias (Cactaceae–Opuntioideae). *Molecular Phylogenetics and Evolution* **65**: 668-681. DOI: https://doi.org/10.1016/j.ympev.2012.07.027

Rosas CMP, Pimienta EB. 1986. Polinización y fase progámica en nopal (*Opuntia ficus-indica* (L.) Miller) tunero. *Fitotecnia* **8**: 164-176.

Rosas U, Barton NH, Copsey L, Reuille PB, Coen E. 2010. Cryptic variation between species and the basis of hybrid performance. *Plos Biology* **8**: e1000429. DOI: https://doi.org/10.1371/journal.pbio.1000429

Rowley GD. 1994. Spontaneous bigeneric hybrids in Cactaceae. *Bradleya* **12**: 2-7. DOI: https://doi.org/10.25223/brad.n12.1994.a2

Russell A, Samuel R, Klejna V, Barfuss MHJ, Rupp B, Chase MW. 2010. Reticulate evolution in diploid and tetraploid species of *Polystachya* (Orchidaceae) as shown by plastid DNA sequences and low-copy nuclear genes. *Annals of Botany* **106**: 37-56. DOI: https://doi.org/10.1093/aob/mcq092

Sang T, Crawford DJ, Stuessy TF. 1997. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). *American Journal of Botany* **84**: 1120-1136. DOI: https://doi.org/10.2307/2446155

Schlumberger BO. 2011. A survey on pollination modes in cacti and a potential key innovation. In: Patiny S. ed. *Evolution of plant-pollinator relationships* (Systematics Association Special Volume Series). Cambridge: Cambridge University Press, pp. 301-319. DOI: https://doi.org/10.1017/CBO9781139014113.011

Schwabe AL, Neale J R, McGlaughlin ME. 2015. Examining the genetic integrity of a rare endemic Colorado cactus *Sclerocactus glaucus* in the face of hybridization threats from a close and widespread congener *Sclerocactus parviflorus.* *Conservation Genetics* **16**: 443-457. DOI: https://doi.org/10.1007/s10592-014-0671-3

Soltis PS. 2013. Hybridization in plants. In: Levin SA. ed. *Encyclopedia of biodiversity* (Second Edition). Waltham: Academic Press, pp. 166-176. DOI: https://doi.org/10.1016/B978-0-12-384719-5.00202-1

Soltis DE, Soltis PS, Tate, JA. 2003. Advances in the study of polyploidy since plant speciation. *New Phytologist* **161**: 173-191. DOI: https://doi.org/10.1046/j.1469-8137.2003.00948.x

Soltis PS, Soltis DE. 2009. The role of hybridization in plant speciation. *Annual Review of Plant Biology* **60**: 561-588. DOI: https://doi.org/10.1146/annurev.arplant.043008.092039
Stebbins GL. 1950. *Variation and evolution in plants*. New York Chichester, West Sussex: Columbia University Press. DOI: https://doi.org/10.7312/steb94536

Stebbins GL. 1971. *Chromosomal evolution in higher plants*. 1st ed. Londres, UK: Edward Arnold Publishers. ISBN: 0713122870

Tapia HJ, Bárcenas-Argüello ML, Terrazas T, Arias S. 2017. Phylogeny and circumscription of *Cephalocereus* (Cactaceae) based on molecular and morphological evidence. *Systematic Botany* **42**: 709-723. DOI: https://doi.org/10.1600/036364417X696546

Tel-Zur N, Mouyal J, Zurigil U, Mizrahi Y. 2020. In support of winge’s theory of “hybridization followed by chromosome doubling.” *Frontiers in Plant Science* **11**: 954 DOI: https://doi.org/10.3389/fpls.2020.00954

Tepedino VJ, Griswold TL, Bowlin WR. 2010. Reproductive biology, hybridization, and flower visitors of rare *Sclerocactus* taxa in Utah’s Uintah Basin. *Western North American Naturalist* **70**: 377-386. DOI: https://doi.org/10.3398/064.070.0310

Trujillo AS, González EM. 1991. Hibridación, aislamiento y formas de reproducción en *Opuntia* spp. *Agrociencia* **1**: 39-58.

Vázquez-Lobo A, Morales GA, Arias S, Golubov J, Hernández-Hernández T, Mandujano MC. 2015. Phylogeny and biogeographic history of *Astrophytum* (Cactaceae). *Systematic Botany* **40**: 1022-1030. DOI: https://doi.org/10.1600/036364415X690094

Vázquez-Sánchez M, Terrazas T, Arias S. 2012. El hábito y la forma de crecimiento en la tribu Cacteae (Cactaceae, Cactoideae). Botanical Sciences 90: 97-108 DOI: https://doi.org/10.17129/botsci.477

Vázquez-Sánchez M, Terrazas T, Arias S, Ochoterena H. 2013. Molecular phylogeny, origin and taxonomic implications of the tribe Cacteae (Cactaceae). *Systematics and Biodiversity* **11**: 103-116. DOI: https://doi.org/10.1080/14772002.2013.775191

Wallace RS, Dickie SL. 2002. Systematic implications of chloroplast DNA sequence variation in the Opuntioideae. *In: Hunt D. ed. Studies in the Opuntioideae (Cactaceae) England: DH Books*, pp. 9-24. ISBN: 9780953813414

Whitney KD, Ahern JR, Campbell LG, Albert LP, King MS. 2010. Patterns of hybridization in plants. *Perspectives in Plant Ecology, Evolution and Systematics* **12**: 175-182. DOI: https://doi.org/10.1016/j.ipees.2010.02.002

Zhang SD, Jin JJ, Chen SY, Chase MW, Soltis DE, Li HT, Yang JB, Li DZ, Yi TS. 2017. Diversification of Rosaceae since the late Cretaceous based on plastid phylogenomics. *New Phytologist* **214**: 1355-1367. DOI: https://doi.org/10.1111/nph.14461

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