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Review / Revisión

The prickly problem of interwoven lineages: hybridization processes in Cactaceae

El espinoso problema de los linajes entrecruzados: procesos de hibridación en Cactaceae

Xochitl Granados-Aguilar[1,2*], Ulises Rosas[2], Antonio González-Rodríguez[3], and Salvador Arias[2]

[1] Posgrado en Ciencias Biológicas, Instituto de Biología, Universidad Nacional Autónoma de México, Ciudad de México, México
[2] Jardín Botánico, Instituto de Biología, Universidad Nacional Autónoma de México, Ciudad de México, México
[3] Instituto de Investigaciones en Ecosistemas y Sustentabilidad, Universidad Nacional Autónoma de México, Morelia, Michoacán, México
*Author for correspondence: xochitl.granados@st.ib.unam.mx

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 Cactaceae presenta muchos géneros con reportes de hibridación.

Preguntas: ¿Cuáles son los patrones reportados de hibridación natural en Cactaceae y sus probables causas? ¿Existen implicaciones filogenéticas y evolutivas relacionadas a 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.

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 those 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 Cacteae (Vázquez-Sánchez *et al*. 2013) and Hylocereeae (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 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.

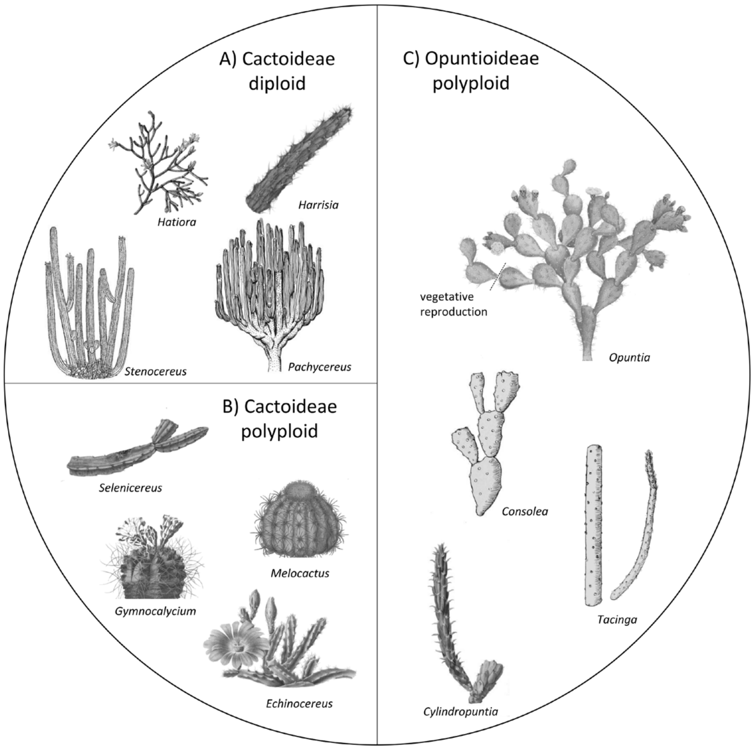

Figure 1. Representative examples of genera with hybridization in Cactaceae. A) Diploid genera from Cactoideae. B) Polyploid genera from Cactoideae. C) Opuntioideae representatives of the genus in the subfamily with reported hybridization. Images modified from Britton & Rose (1919, 1920, 1922, 1923) and Arias *et al.* 2012.

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 incompatible 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 & Pimienta 1986, Anderson 2001, Schlumpberger 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*, *Myrtillocactus*, *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 Interspecific=a Intergeneric=b**	**No. of naturally occurring hybrids**
Cactoideae	Echinocereeae	8	a, b	9
	Hylocereeae	2	a	6
	Cereeae	6	a, b	13
	Trichocereeae	14	a, b	16
	Cacteae	7	a, b	6
	Notocacteae	-	-	-
	Rhipsalideae	-	-	-
Opuntioideae	Cylindropuntieae	1	a	11
	Opuntieae	3	a, b	38
	Austrocylindropuntieae	-	-	-
	Pterocacteae	-	-	-
	Tephrocacteae	-	-	-
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 Echinocereeae or Cereeae due to their long generational 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). Hybridization includes other processes by which new species can originate. 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 hybridization 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 autopolyploids 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 *Weberbauerocereus*, 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 & Mrâĉek 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 parentals 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. 1 A 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 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 F_1 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 parentals 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 *Brasiliopuntia*, *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 autopolyploid 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 Basilares, Scheerianae 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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