






Can plant hybridization and polyploidy lead to pollinator shift?

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ABSTRACT

Events of both hybridization and polyploidy are capable of completely restructuring the genome, modifying phenotypic traits and affecting ecological interactions. For plants, these changes may affect floral traits that are important for interactions with pollinators, which could lead to shifts in pollinator behavior and taxa between hybrids/polyploids and parental/diploid species. Such pollinator shifts have great ecological and evolutionary relevance since they play a key role in the diversification of angiosperms. There is a growing number of studies that explicitly address the relationship between plant hybridization/polyploidy and pollinator shifts. However, questions remain about how often hybridization and polyploidy lead to pollinator shifts and what are the mechanisms that mediate this process. We reviewed studies that compared the reproductive biology of hybrids/polyploid with that of parental/diploid species. These studies are based on modifications of floral traits involved in attracting and rewarding pollinators. We also discussed how such changes in flower traits are widespread among plant taxa and affect pollinator visitation rates, pollinator fidelity, pollen movement, and could lead to pollinator shifts. All of these consequences are underexplored, especially from the perspective of pollinators, which foster future research that integrates genetics, ecology, and evolution of plant-pollinator interactions.

Keywords: allopolyploidy, autopolyploidy, floral traits, hybrid species, homoploid, pollination

Introduction

The composition and diversity of floral traits are considered one of the main factors mediating plant-pollinator interactions (Shuttleworth & Johnson 2010; Rosas-Guerrero *et al.* 2014; Bergamo *et al.* 2017; Ramos & Schiestl 2019). Such traits include flower size, shape, color, and scent, as well as rewards offered to pollinators, such as nectar, pollen, essential oils and resins (Simpson & Neff

1981; Willmer 2011; Schiestl & Johnson 2013; Agostini *et al.* 2014). Besides the influence of different ecological interactions (Barber & Gorden 2015; Lucas-Barbosa 2015; Ramos & Schiestl 2019), the floral traits are also affected by environmental conditions through the resource availability and allocation for plant reproduction (Obeso 2002; Strauss & Whittall 2006; Sletvold *et al.* 2017), and by genetic factors that drive the floral development (Coen & Meyerowitz 1991; Soltis 2002; Hermann *et al.* 2015; Smith 2016). The flower morphology and rewards are regulated by specific

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genes, the ABCDE system (Baum *et al.* 2001; Bowman *et al.* 2012; Morel *et al.* 2018), but they are also subject to events that change the whole genome, affecting gene expression and, by consequence, the floral phenotype and ecological interactions, such as pollination (Vamosi *et al.* 2007; Chase *et al.* 2010; Pegoraro *et al.* 2016; Segreaves 2017; Pegoraro *et al.* 2019; Porturas *et al.* 2019). Therefore, to better understand the evolutionary changes in floral traits and how variation in the plant genome may affect plant-pollinator interactions, an integrated approach connecting genetic factors and pollinator responses is necessary.

Hybridization and polyploidy are among the main sources of genome variation in plants (Wood *et al.* 2009; Soltis *et al.* 2014; 2015; Clark & Donoghue 2018). While the former promotes a mixture of two genomes in a single nucleus after an inter-specific or inter-generic crossing, the latter consists of the whole genome duplication (*i.e.*, the state of having more than two complete sets of pairing chromosomes; for definitions see Stebbins 1971; Soltis & Soltis 2009; Schubert & Vu 2016; Van der Peer *et al.* 2017; Pelé *et al.* 2018). The success of the hybridization depends on the evolutionary divergence between progenitors. The Darlington's rule predicts that, in order to the hybridization event to succeed, the two parental genomes should not be so divergent, to facilitate the subsequent process of diploidization, but, on the other hand, not so similar, to avoid the irregular meiotic multivalent formation which

reduces the hybrid fertility (Winge 1917; Darlington 1937; but see Buggs *et al.* 2011; Schubert & Vu 2016; Wagner *et al.* 2019). Polyploidy, in turn, represents one of the most dramatic mutations in eukaryotes (Otto 2007), and it is considered one of the main mechanisms of angiosperms evolution (Schubert & Vu 2016; Van der Peer *et al.* 2017; Clark & Donoghue 2018).

Polyploidy is commonly originated by one of the two main pathways (Otto & Whitton 2000; Pelé *et al.* 2018): (1) hybridization, when the combination of the two genomes is followed by genome duplication through the formation of non-reduced gametes. The originated individuals are called allopolyploids with $2n = 4x$ ("x" indicate the base chromosome number; Thompson & Lumaret 1992; Soltis *et al.* 2015; see Fig. 1A); (2) fusion of two non-reduced gametes from the same species originating autopolyploid individuals with $2n = 4x$ (Fig. 1B). The unreduced gametes are generated by the first or second meiotic division restitution, *i.e.*, restitution nuclei in meiosis I or II that generates gametes with diploid number of chromosomes, as first stated by Harlan & de Wet (1975) (but see also Appels *et al.* 1998; Ramsey & Schemske 1998; Pelé *et al.* 2018). Nevertheless, the non-reduced gametes could also be originated from somatic doubling in stems with flowers, but it seems to be more frequent in crop species (Ramsey & Schemske 1998; Otto & Whitton 2000).

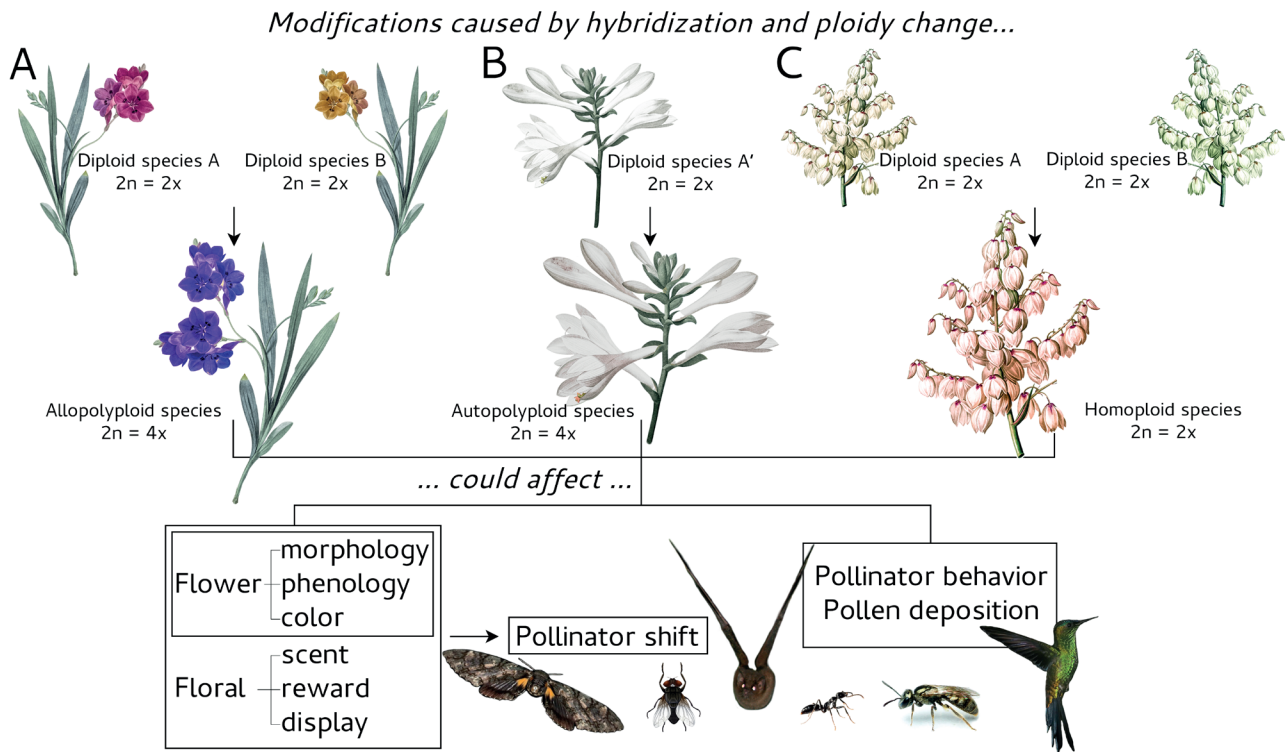


Figure 1. A. Allopolyploidy, B. Autopolyploidy, C. Homoploidy. Below follow the main floral traits affected by hybridization and polyploidy and the main traits affected in the pollinator taxa (in the absence of pollinator shift). Figures were modified from Freepik (<https://www.freepik.com/>), except by hummingbird, bat, and hawkmoth photos, which were supplied by F.W. Amorim.

It is suggested that all angiosperm lineages, including fossil groups, had experienced at least one event of whole genome duplication during its evolutionary history (Jiao *et al.* 2011; Wendel 2015; Magallón *et al.* 2019), and that about 25 % of all vascular plants are recent allopolyploids (Mallet 2007). Moreover, it is stated that at least 15 % of angiosperm speciation events are followed by ploidy increase (Wood *et al.* 2009). Concerning the natural systems, allopolyploids is traditionally assumed to be more frequent than autopolyploids (but see Ramsey & Schemske 1998); nevertheless, autopolyploids may be as frequent as allopolyploids since the former could be a neglected subject in taxonomic studies (Soltis *et al.* 2007; Parisod *et al.* 2010; Barker *et al.* 2016).

However, not all hybrids are necessarily polyploids. At least 30 hybrid plant species have been described as homoploid hybrids: an interspecific hybrid species that did not undergo polyploidy (see Buerkle *et al.* 2000; Gross & Reiseberg 2005; Mallet 2007; Soltis & Soltis 2009; Abbott *et al.* 2013). Due to the absence of genome duplication, the homoploids present $2n = 2x$, as a diploid species (Fig. 1C). During homoploid hybrid meiosis, a pairing between homeologous chromosomes (*i.e.*, “homologous” chromosome, but inherited from different parental species) may occur and restore the fertility without polyploidization (Rieseberg & Willis 2007). However, due to the absence of polyploidization as an instantaneous reproductive barrier, the homoploid genome may remain porous for a longer period and speciation tend to be a more time-consuming process (Buerkle *et al.* 2000; Yakimowski & Reiseberg 2014; Ma *et al.* 2016). Thus, although hybridization and polyploidization are often considered related processes, they can also occur independently, leading to different outcomes in plant phenotype.

After the hybridization and/or polyploidization event, the diploidization process takes place so the allo-/autopolyploid genome is completely reorganized to work as a diploid one (Bossdorf *et al.* 2008; Dodsworth *et al.* 2016; Peer *et al.* 2017; Jiao 2018). This process favors numerous genome modifications, such as intense retrotransposon activation, chromosome rearrangements, and epigenetic changes that may represent genome constraints for the establishment of the newly formed hybrids in the population. Besides these constrain, the new hybrids need also to overcome the minority cytotype exclusion (first defined by Levin 1975), since they are usually rare and the poor availability of genetically compatible partners for reproduction can hamper their reproductive success (Abbott *et al.* 2013; Suda & Herben 2013; Casazza *et al.* 2017; Pegoraro *et al.* 2016; 2019; Porturas *et al.* 2019).

During the first steps of the polyploid establishment, triploids individuals are frequently formed (Fig. 2). However, since the triploid progeny is usually sterile (*i.e.*, the majority of triploid gametes are unbalanced and nonviable), an instantaneous reproductive barrier between the diploid

parental and the polyploid hybrid is formed (*i.e.*, the triploid block, Fig. 2A). Nevertheless, it has been currently observed that triploids individuals could generate a small number of balanced gametes (x , $2x$ or $3x$ gametes) and, considering such euploid gametes, the triploid individuals, by crossing with diploid and tetraploid ones, could generate a viable progeny (Fig. 2B - C). This event, known as a *triploid bridge*, generates new polyploid progenies, favoring the establishment of the polyploid population, but also keeping the genome porous by the crossing with diploid individuals (reviewed by Ramsey & Schemske 1998; Coyne & Orr 2004; Husband 2004, see also Moraes *et al.* 2013; Pegoraro *et al.* 2016). In this sense, the subsequent speciation process will depend on the counteracting of genetic and ecological breeding barriers, besides increasing the probability of successful intra-cytotype mating (Gross & Schiestl 2015). Assortative mating with compatible partners mediated by the interaction with pollinators may lead to reproductive isolation between hybrids and their progenitors through pre- and postzygotic barriers and favor the long-term hybrid establishment (Kay 2006; Sun *et al.* 2015; Kostyun & Moyle 2017).

The ecological divergence between parental plants and the new hybrids configures a crucial isolation mechanism. Generally, hybrid plants (both polyploid and homoploid) present differences in the geographic distribution compared with their parental plants, even without great differences in climatic conditions and pollination niches (Stebbins 1959; Thompson *et al.* 2004; Thompson & Merg 2008; Rentsch & Leebens-Mack 2012; Vallejo-Marin & Hiscock 2016; Casazza *et al.* 2017). Moreover, some hybrids are incapable to survive at the parental habitat and vice-versa (*e.g.*, *Helianthus* homoploid hybrids; Gross & Reiseberg 2005). Such a difference in the geographic distribution pattern of hybrids is probably related to the phenotypic changes derived from the new genome constitution. These changes may affect plant reproductive characteristics, such as the sexual system, with a positive association between ploidy and dioecy (Miller & Venable 2000; Glick *et al.* 2016), ploidy and asexual reproduction, especially apomixis (Otto & Whitton 2000; Hojsgaard & Hörandl 2019), and ploidy and self-compatibility, with hybrids derived from obligatory outcrossing parental species becoming self-compatible (Abbott & Lowe 2004; Lowe & Abbott 2004; Mable 2004; Barringer 2007). Baker's law states that self-compatibility along with the autonomous reproduction by spontaneous self-pollination and the asexual reproduction by apomixis play an important role in the establishment of new polyploid hybrid species beyond the range of parental species original distribution (Pannell *et al.* 2015; Schinkel *et al.* 2016).

Polyploidy seems also to affect floral traits associated with pre-mating barriers between polyploids and diploids, especially when these changes are related with phenology and morphology (Pegoraro *et al.* 2016; 2019; Porturas *et al.* 2019), whose affect plant-animal interactions



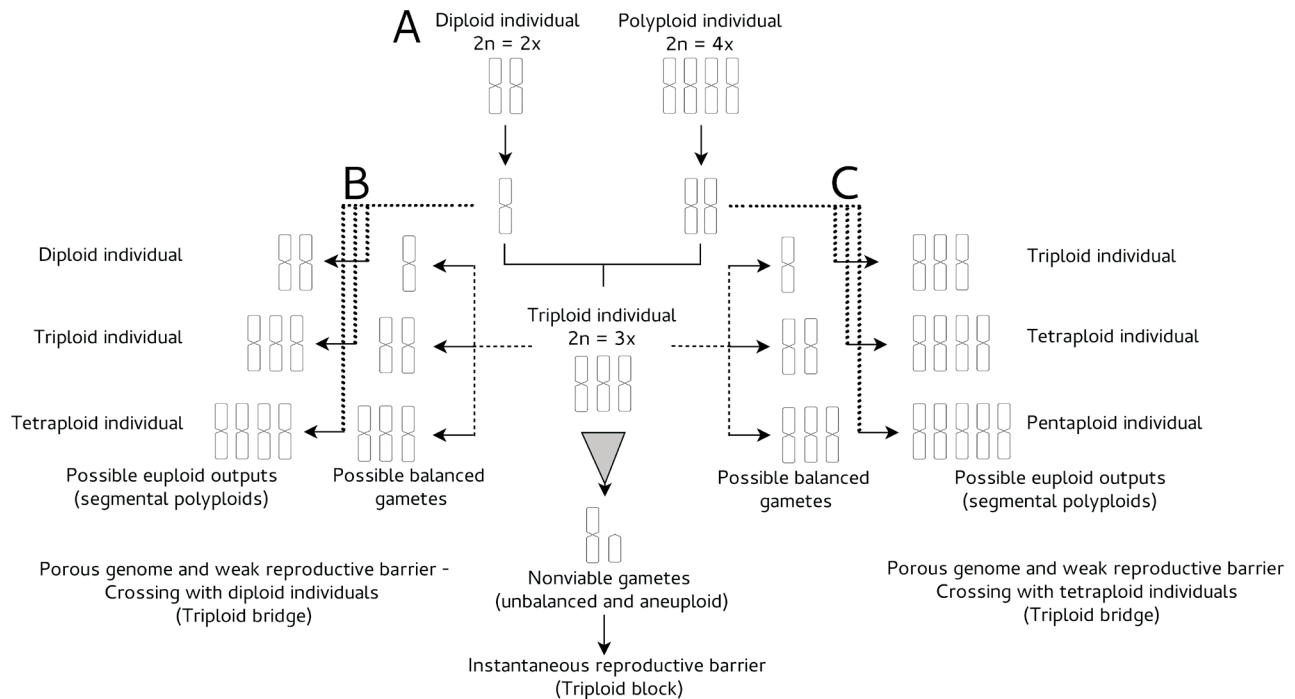


Figure 2. The triploid block and bridge. **A.** The triploid block (from top to bottom). The interploidal crossing between diploid and tetraploid individuals produce a triploid progeny, that is commonly sterile (*i.e.*, produce nonviable gametes, see the large arrow), resulting in an instantaneous reproductive barrier. **B.** and **C.** The triploid bridge. However, triploid individuals could also produce a few balanced gametes (see dashed lines) with one, two or three chromosomes for each chromosome pair. The backcrossing of these triploid balanced gametes with the balanced gametes generated by the diploid and tetraploid individuals (see dotted lines) could produce polyploid offspring, increasing the frequency of polyploid individuals in the population. As a result, it will help the newly tetraploid individuals to overcome the minority cytotype exclusion. Large gray arrow in **A** indicates the majority of the generated triploid gametes. Dashed lines to **B** and **C** indicate the few balanced gametes generated by the triploid individuals. Dotted lines in **B** and **C** indicated the possible crossings between balanced triploid gametes and balanced diploid (**B**) and tetraploid (**C**) gametes.

(Segraves & Anneberg 2016; Ramos & Schiestl 2019). Since pollinators may respond to small variations in floral signals and rewards (Junker *et al.* 2013), changes in floral traits following hybridization and polyploidization may affect plant-pollinator interactions by modifying pollinator taxon, behavior, morphological match with floral parts, and thus the selection regime (Nuismer & Cunningham 2005; Thompson & Merg 2008; Cortis *et al.* 2009; Chase *et al.* 2010; Svensson *et al.* 2016). These ecological consequences may be strongly context-dependent, once they are subjected to the availability of pollinators in the community, as well as their requirements, *i.e.* pollinator preferences that affect floral choice behavior and visitation pattern (Shuttleworth & Johnson 2010; Vereecken *et al.* 2010; McCarthy *et al.* 2016). Nevertheless, the overall impact of changes in floral traits that affect plant-pollinator interactions will also correspond to the degree of plant dependence on pollinators for pollen exchange and sexual reproduction (Whitney & Glover 2007).

Given all the possibilities of flower modifications that follow plant hybridization and/or polyploidy, there are promising paths for studies that address the evolution of floral traits in hybrids and polyploids through an ecological perspective, especially addressing plant-pollinator

interactions. Previous studies reported the consequences of hybridization and polyploidization on plant-pollinator interactions (Vamosi *et al.* 2007; Barker *et al.* 2016; Ma *et al.* 2016; Segraves & Anneberg 2016), but some important gaps in the knowledge remain, especially regarding how often hybridization and polyploidization could be associated with pollinator shift, and what are the main floral traits related to this process. Aiming to better understand this scenario and guide new studies, here we review the literature that explicitly addresses plant-pollinator interactions following hybridization and polyploidization events. We present an overview of studies on this topic and summarize their conclusions about the mechanisms by which hybridization and polyploidization may or may not lead to pollinator shift. We hope to highlight the opportunities for future research on this neglected topic and bridge an important gap between plant genetics and plant-pollinator interactions.

Materials and methods

We performed a literature survey from March / 2018 to December / 2019 at the online databases of Google Scholar and Web of Science for all studies published from 1955

until 2019. We used a combination of the search topics: “plant polyploid*”, “plant hybrid*”, “pollinat* shift” and “pollinat*”, where “*” states for different suffixes. We also reviewed the literature cited in the selected articles. For the synthesis, we selected those studies that: (1) explicitly address the relationship between hybridization and/or polyploidy with pollination, not only with floral traits; and (2) that were conducted in natural rather than crop systems, given the incidence of genetic manipulation in crop systems to enhance plant productivity (for review see Hojsgaard & Hörandl 2019). We did not select studies based on only one aspect of the changes in pollination (e.g. pollinator taxa shift) to assess the broad aspects of such effects (e.g. pollinator behavior changes). Likewise, we did not select studies on polyploid hybrids only, to include studies in which the effect of hybridization on pollination was assessed regardless of polyploidy, that is, in homoploid plants. All taxonomic names were checked and updated when necessary on the online datasets of The Plant List and Taxonomic Name Resolution Service (Boyle *et al.* 2013). The hybrids that did not receive any taxonomic treatment until now were cited by the progenitor names separated by “x” following the International Code of Botanical Nomenclature (ICBN) (Turland *et al.* 2018). If the plant species is a hybrid or not was based on the information from the literature. The ploidy level (the number of complete duplications of the chromosome set) was indicated based also on the literature or from the Chromosome Count Data Base (CCDB; Rice *et al.* 2015, available at <http://ccdb.tau.ac.il/>).

Results

Our survey resulted in 37 papers with reports for 37 plant taxa distributed over 18 genera from 13 families (Tab. 1). Overall, 28 out of the 37 taxa (75.7 %) were considered hybrids (homo- or allopolyploid), while nine taxa (24.3 %) were autopolyploids. Pollinator shift between hybrids and progenitors was reported for 21 taxa (75 % out of 28), which was constant when considering just homoploids (nine of the 12 taxa) and when considering the allopolyploids (12 out of the 16 taxa). Among autopolyploids, pollinator shift was detected for only a single taxon (11.1 % out of nine taxa, Tab. 1). Pollinator shift was followed by differences in at least one floral trait between hybrid and parental plants, and autopolyploid and diploid plants. Among hybrids without pollinator shift, we found a case in the bromeliad genus *Pitcairnia* sp., which the hybrid is pollinated by hummingbirds as its parental species (Wendt *et al.* 2001), besides the case of *Primula marginata*, which could, possibly, partially share the pollinator assemblage with its parental (Casazza *et al.* 2017). For the hybrid orchids from the genera *Dactylorhiza* (De Hert *et al.* 2011; 2012) and *Epidendrum* (Moraes & Amorim, personal observations), both presenting pollination by deceit, the hybrids present the same pollinators as the parental species. Finally, the last

case involved the hybrid *Yucca gloriosa* that share pollinators with its parentals, despite the high pollinator specificity commonly observed among species of the *Yucca* genus (see Rentsch & Leebens-Mack 2012).

Regarding floral modification, the most frequent flower traits reported to be affected by hybridization and polyploidy was floral morphology (83.8%), followed by color (59.46%) and phenology (27.02%, Tab. 1 and Fig. 1). These characteristics are in the main set of floral traits that mediate the communication between plants and pollinators, along with floral scent (changed by hybridization and polyploidy in 21.6% of the reports), the amount and quality of floral rewards such as nectar, and the number of flowers (*i.e.* floral display) (both changed by hybridization and polyploidy in 5.4% of the reports – Tab. 1 and Fig. 1).

Discussion

Effects of hybridization and polyploidy on floral traits

The floral morphology modifications associated with hybridization and polyploidy could be detected in petals size and shape, length and mouth width of the floral tube, besides style and anthers shape (examples in Fig. 3). Floral size may be incorporated in the measures of morphology since this modification is hard to dissociate from flower shape (but see the case of *Gymnadenia conopsea* cytotypes in Gross & Schiestl 2015). Considering the magnitude of the difference in floral size (*i.e.*, the intensity of the effect) and the trend for increase or decrease in floral size (*i.e.*, the direction of the effect), the studies reviewed usually did not discuss intensity, but it seems to be a consensus about the direction of the effect: polyploid species generally have larger flowers than diploids, as detected for *Anacamptis pyramidalis*, *Epilobium angustifolium*, *Gymnadenia conopsea*, *Heuchera grossulariifolia*, *Libidibia ferrea* (Tab. 1), and the allopolyploid *Emilia forsbergii* (Moraes *et al.* 2010, compare the flowers of the allopolyploid *E. forsbergii* and the diploid parental *E. sonchifolia* in Fig. 3A-B). Such increase in size reflects the ‘giga effect’, in which an increase in genome size, caused by polyploidy, is reflected in the cell size, which is enlarged and is correlated with the increase in the size of several plant organs, as guard-cells in the stomata and the pollen grains (Stebbins 1971; Katsiotis & Forsberg 1995; Levin 2002; Knight & Beaulieu 2008; but also compare leaf sizes in Fig. 3A-B).

Changes in flower size affect the communication between flower and pollinator and also the pollinator behavior and accessibility to the flower. These modifications can specifically affect the accessibility of the pollinator to the floral reward, compromising the plant-pollinator match. For example, in the dodecaploid *Primula marginata*, hybridization affected the floral tube length (increase) and mouth width (intermediate between hexaploid parentals), suggesting that the hybrid could share a subset of parental



Table 1. Synthesis of the plant ploidy, hybridization, floral traits change, and pollinator shift based on the literature review. The ploidy level (the number of complete duplications of the chromosome set) is indicated as “2n” for diploid, “3n” for triploid, “4n” for tetraploid, “6n” for hexaploid and “8n” for octaploid. Following the ICBN (Turland *et al.* 2018), the hybrids that did not receive any taxonomic treatment until now are cited by the progenitor names separated by “×”. In the case of the two *Narcissus* species and *Nicotiana* × *obtusata* Krügel, the “×” indicates a hybrid origin for these taxa that already received new names. Polyploid types are expressed as Auto (autopolyploid), Homo (homoploid), and Allo (allopolyploid). Floral traits are expressed as Morph (morphology), Pheno (phenology), Frag (fragrance), Disp (display), and Nect (nectar). Pollinator shift refers to a shift in pollinator taxa between hybrid and progenitor / diploid and polyploid plants. The “-” indicates data not specified in the study

Plant taxon	Ploidy	Hybrid	Origin	Floral trait change	Pollinator shift	Reference
Amaryllidaceae J. St.-Hil. (One genus, two hybrid species)						
<i>Narcissus</i> × <i>alentejanus</i> Fern. Casas	-	Yes	Homo ¹	Morph, Frag, Nect	Yes	Marques <i>et al.</i> 2016
<i>Narcissus</i> × <i>perezlarae</i> Font Quer	2n	Yes	Homo	Morph, Frag, Nect	Yes	Marques <i>et al.</i> 2016
Asparagaceae Juss. (One genus, two species)						
<i>Yucca brevifolia</i> Engelm.	2n	Yes	Homo	Morph, Frag	Yes	Smith <i>et al.</i> 2009; Svensson <i>et al.</i> 2016
<i>Yucca gloriosa</i> L.	2n	Yes	Homo	Morph	No	Rentsch & Leebens-Mack 2012
Asteraceae Bercht. & J. Presl (One genus, one species)						
<i>Aster amellus</i> L.	6n	No	Auto	Pheno	No	Castro <i>et al.</i> 2011
Bromeliaceae Juss. (One genus, one hybrid entity)						
<i>Pitcairnia albiflos</i> × <i>Pitcairnia staminea</i>	-	Yes	Allo ²	Morph, Pheno, Frag, Color	No	Wendt <i>et al.</i> 2001
Iridaceae Juss. (One genus, one species)						
<i>Iris nelsonii</i> Randolph	2n	Yes	Homo	Morph, Color	Yes	Emms & Arnold 2000; Taylor <i>et al.</i> 2012; Taylor <i>et al.</i> 2013
Fabaceae Lindl. (Two genera, two species)						
<i>Acacia mangium</i> Willd.	4n	No	Auto	Morph, Pheno	No	Nghiem <i>et al.</i> 2011
<i>Libidibia ferrea</i> (Mart. Tu.) L.P.Q.	4n	No	Auto	Morph	No	Borges <i>et al.</i> 2012
Liliaceae Juss. (One genus, one species)						
<i>Erythronium albidum</i> Nutt.	4n	No	Auto	Pheno	No	Roccaforte <i>et al.</i> 2015
Onagraceae Juss. (One genus, one species)						
<i>Epilobium angustifolium</i> L. (cited as <i>Chamerion angustifolium</i> (L.) Holub)	4n	No	Auto	Morph, Pheno	No	Husband & Schemske 2000; Husband & Sabara 2003; Kennedy <i>et al.</i> 2006
Orchidaceae Juss. (Five genera, four species and four hybrid entities)						
<i>Anacamptis pyramidalis</i> (L.) Rich.	4n	No	Auto	Morph, Pheno	Yes ³	Pegoraro <i>et al.</i> 2016; Pegoraro <i>et al.</i> 2019
<i>Dactylorhiza praetermissa</i> (Druce) Soó	4n	Yes	Allo	Morph	No ³	Hert <i>et al.</i> 2011
<i>Dactylorhiza incarnata</i> (2n) × <i>D. praetermissa</i> (4n)	3n, 6n	Yes	Allo	Morph	No ³	Hert <i>et al.</i> 2012
<i>Epidendrum fulgens</i> × <i>Epidendrum puniceolutesum</i>	3n	Yes	Homo	Color	No ³	Pinheiro <i>et al.</i> 2010; Moraes <i>et al.</i> 2013
<i>Epidendrum fulgens</i> × <i>Epidendrum denticulatum</i>	3n	Yes	Homo	Morph, Color	No ³	Pinheiro <i>et al.</i> 2015
<i>Gymnadenia conopsea</i> (L.) R. Br.	4n	No	Auto	Frag, Color, Disp	No	Gross & Schiestl 2015
	8n	No	Auto	Morph, Pheno, Frag	No	Jersáková <i>et al.</i> 2010
<i>Gymnadenia densiflora</i> A. Dietr.	4n	No	Auto	Pheno, Frag	No	Jersáková <i>et al.</i> 2010



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Table 1. Cont.

Plant taxon	Ploidy	Hybrid	Origin	Floral trait change	Pollinator shift	Reference
<i>Ophrys arachnitiformis</i> × <i>Ophrys lupercalis</i>	3n	Yes	Allo	Frag	Yes	Ayasse 2006; Vereecken <i>et al.</i> 2010
Plantaginaceae Juss. (One genus, two species)						
<i>Penstemon spectabilis</i> Thurb. ex A. Gray	2n	Yes	Homo	Morph, Color	Yes	Wolfe <i>et al.</i> 1998
<i>Penstemon clevelandii</i> A. Gray	2n	Yes	Homo	Morph, Color	Yes	Straw 1955; Straw 1956; Wolfe <i>et al.</i> 1998
Primulaceae (One genus, one species)						
<i>Primula marginata</i>	12n	Yes	Allo	Morph, Color, Pheno	No	Casazza <i>et al.</i> 2017
Saxifragaceae Juss. (One genus, one species)						
<i>Heuchera hallii</i> var. <i>grossularifolia</i> (Rydb.) Rosend (cited as <i>Heuchera grossulariifolia</i> Rydb.)	4n	No	Auto	Morph, Pheno, Disp	Yes	Seagraves & Thompson 1999; Nuismer & Cunningham 2005; Thompson & Merg 2008
Solanaceae Juss. (One genus, 14 species)						
<i>Nicotiana arentsii</i> Goodsp.	4n	Yes	Allo	Morph, Color	Yes ⁴	Chase <i>et al.</i> 2010; McCarthy <i>et al.</i> 2015; 2016
<i>Nicotiana clevelandii</i> A. Gray	4n	Yes	Allo	Morph, Color	Yes ⁴	
<i>Nicotiana nesophila</i> I.M. Johnst.	4n	Yes	Allo	Morph, Color	Yes ⁴	
<i>Nicotiana nudicaulis</i> S. Watson	4n	Yes	Allo	Morph, Color	Yes ⁴	
<i>Nicotiana</i> × <i>obtusata</i> Krügel	4n	Yes	Allo	Morph, Color	Yes ⁴	
<i>Nicotiana quadrivalvis</i> Pursh	4n	Yes	Allo	Morph, Color	Yes ⁴	
<i>Nicotiana repanda</i> Lehm.	4n	Yes	Allo	Morph, Color	Yes ⁴	
<i>Nicotiana rustica</i> L.	4n	Yes	Allo	Morph, Color	Yes ⁴	
<i>Nicotiana suaveolens</i> Lehm.	4n	Yes	Allo	Morph, Color	Yes ⁴	
<i>Nicotiana stocktonii</i> Brandege	4n	Yes	Allo	Morph, Color	Yes ⁴	
<i>Nicotiana tabacum</i> L.	4n	Yes	Allo	Morph, Color	Yes ⁴	
<i>Nicotiana glauca</i> Graham	2n	Yes	Homo	Morph, Color	Yes ⁴	
<i>Nicotiana linearis</i> Phil.	2n	Yes	Homo	Morph, Color	Yes ⁴	
<i>Nicotiana glutinosa</i> L.	2n	Yes	Homo	Morph, Color	Yes ⁴	

¹The chromosome number for *Narcissus* × *alentejanu* Fern. Casas is unknown and the homoploid condition is suggested from the information that this hybrid is very similar to *Nicotiana* × *perezlarae* (2n=29) generated from the same parents - *Nicotiana serotinus* L. (2n=30) and *Narcissus obsoletus* (Haw.) Spach (2n=22). Chromosome numbers were verified at CCDB (Rice *et al.* 2015).

²There is no information about the chromosome number of this hybrid of *Pitcairnia* and we assume it as an allopolyploid, since it is much more common than homoploid and there is no homoploid described for Bromeliaceae.

³Food deceptive pollination system. The pollination service is guaranteed by naïve insects.

⁴Pollinator shifts are estimated by changes in floral morphology and color spectra and not by empirical observation.



pollinators (Casazza *et al.* 2017). This is based on the assumption that plants with shorter and wider mouth flower tubes could interact with a greater diversity of pollinators compared to plants with long and narrow floral tubes, as observed in plants pollinated by hawkmoths, butterflies, and flies (Fenster *et al.* 2004; Moré *et al.* 2012; Borba *et al.* 2011; Amorim *et al.* 2014; Pisano *et al.* 2019).

The giga effect could also be detected among homoploid hybrids. Examples include the homoploids *Narcissus* sp., which presents an increase in floral tube mouth in comparison to the parental plants (Marques *et al.* 2016); *Nicotiana glauca* and *Nicotiana linearis*, for which the floral limb size is larger in comparison to progenitors (McCarthy *et al.* 2016); and also the hybrid between *Epidendrum denticulatum* × *Epidendrum fulgens* (Pinheiro *et al.* 2015; Fig. 3C). In these cases, the giga effect is not a result of genome size increase but should be a result of the heterosis, also known as hybrid vigor (Birchler *et al.* 2006; Govindaraju 2019). The heterosis observed in hybrids is usually expressed in structures associated with reproductive success, suggesting that it was selected over the evolutionary time and may favor the new hybrid reproduction (Birchler *et al.* 2006).

Another floral trait often changed under the effect of polyploidy is the floral phenology (*i.e.*, the difference in flowering time), which may act as a reproductive barrier regardless of pollinator shift and potential changes in the plant reproductive system (Tab. 1 and Fig. 1). For example, in the autopolyploid *Heuchera grossulariifolia* (Segraves & Thompson 1999; Thompson & Merg 2008) and the allopolyploid *Senecio eboracensis* (an interspecific tetraploid species originated from the crossing *S. vulgaris* × *S. squalidus*; Abbott & Lowe 2004), both diploid and polyploid individuals occur in sympatry and shifts in flowering phenology created a reproductive barrier. Such phenology difference was considered one of the main reproductive barriers between diploid and autotetraploid populations of *Anacamptis pyramidalis*, along with microhabitat and triploid sterility, ensuring the establishment of a strong reproductive barrier between cytotypes, even when sharing pollinators (Pegoraro *et al.* 2019). Changes in floral phenology enable plants to explore new pollination niches, eventually leading to reproduction isolation (Thompson & Lumaret 1992). Such effect, along with changes in flower size, could be exemplified by the autopolyploid *Heuchera grossularifolia*, in which diploids (with smaller flowers)

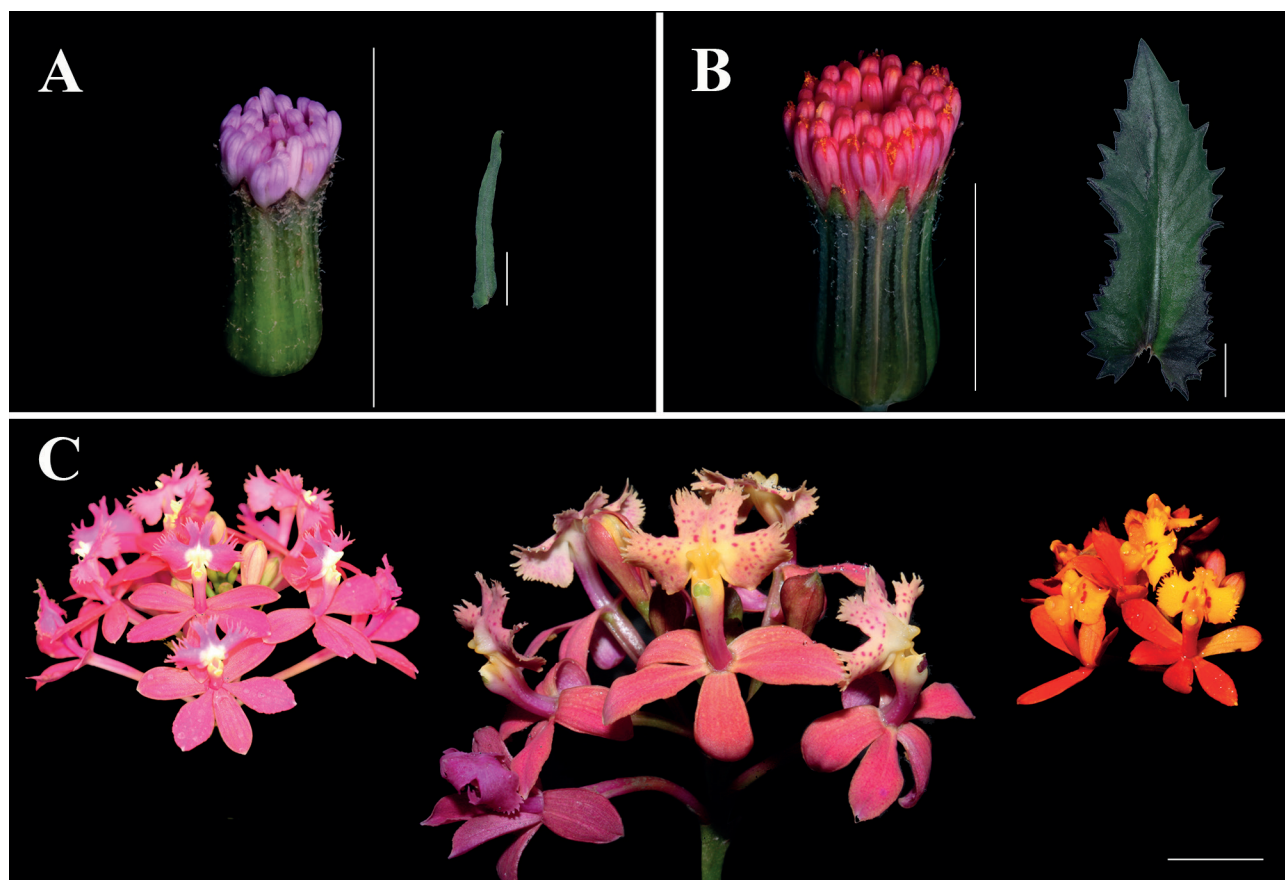


Figure 3. Morphological changes between parental diploid species and polyploidy / homoploid hybrids. **A.** Flower and leaf of the diploid *Emilia sonchifolia* ($2n = 2 \times = 10$), **B.** Flower and leaf of the allotetraploid *Emilia fosbergii* ($2n = 4 \times = 20$), **C.** *Epidendrum denticulatum*, the hybrid *Epidendrum fulgens* × *Epidendrum denticulatum* and *Epidendrum fulgens*, respectively from left to right. Bars indicate 1 cm. All photos by A.P. Moraes.

were most visited by worker bees of the Halictidae family, while the autopolyploids (larger flowers) were most visited by queen bees, moths and flies of the Bombyliidae family (Segraves & Thompson 1999).

The color differences are relatively common between hybrids and progenitor / diploid and polyploid plants (Wendt *et al.* 2001; McCarthy *et al.* 2015; Fig. 1), as could be observed comparing the hybrid *Epidendrum denticulatum* × *Epidendrum fulgens* and its parental (see Fig. 3C; Pinheiro *et al.* 2015). However, few studies contextualized this difference concerning the interaction with pollinators and ploidy (see Teixeira *et al.* 2020) and here, the high prevalence of color changes (59.46 % of the records) could have a bias due to the *Nicotiana*, with all 14 species presenting color variation and pollinator shift. Such contextualization is more explicit for floral scent, even considering that changes in floral scent seem to be less frequent than color changes (Tab. 1). The floral scents in hybrids usually present one of the three possible outputs when compared with parental species: (1) an intermediate chemical profile, (2) absence of some compounds or (3) a transgressive profile, with synthesis of novel compounds not emitted by either parental species (Pisano *et al.* 2019). For example, in the orchid *Ophrys* and the *Narcissus*, as well as the supposed interspecific between *Mandevilla laxa* × *M. pentlandiana*, the hybrids emitted different volatile compounds compared to their parental (*i.e.*, transgressive profile), affecting the behavior of common pollinators and attracting new floral visitors (Vereecken *et al.* 2010; Ma *et al.* 2016; Marques *et al.* 2016; Pisano *et al.* 2019). The *Narcissus* hybrids showed also a change in floral rewards, with an increase in nectar production associated with the emergence of ant pollination (Marques *et al.* 2016).

Changes in flower morphology, phenology, color, and scent, besides to be a consequence of hybridization, could promote reproductive isolation by pre-zygotic barrier even among plants sharing the same pollinators (Cortis *et al.* 2009; Castro *et al.* 2011, see also Niet & Johnson 2012; Niet *et al.* 2014; Teixeira *et al.* 2020). Besides, it should be noticed that changes in floral traits may also affect the pollinator behavior and pollen deposition on the pollinator body (Fig. 1). Thus, even without pollinator shift, there may be a change in the pollination outcome after plant hybridization and polyploidy. These possibilities are better discussed below.

Effects of hybridization and plant polyploidy on pollinators

The effect of plant hybridization and polyploidy on plant-pollinator interactions was reported in plants pollinated by several insect taxa, such as Hymenoptera, Lepidoptera, Diptera, Coleoptera, and Heteroptera, as well as by vertebrates, like hummingbirds (Tab. 1). However, in many cases, hybrids interacted with more than one pollinator taxa and the pollinator shift involved a taxon already interacting

with the progenitor plants, as for *Nicotiana* spp. (Chase *et al.* 2010), or required a new pollinator taxon, as for *Narcissus × alentejanus* (Marques *et al.* 2016), *Ophrys* (Vereecken *et al.* 2010) and *Heuchera grossularifolia* (Segraves & Thompson 1999; see discussion in the previous section).

Although all cases of pollinator shift assessed here were accompanied by some level of change in floral traits, the opposite was not true. There are at least three cases in which floral traits are different between diploid and polyploid/parental and hybrid species without pollinator shift: the legumes *Acacia mangium* and *Libidibia ferrea*, and the orchid *Gymnadenia conopsea* (Tab. 1). In the first two cases, there was a change in floral phenology, which could potentially lead to reproductive isolation among plants without pollinator shift. Moreover, there were examples involving changes in floral traits with effects on pollinator behavior, but not on pollinator identity (Fig. 1). This happened to the autopolyploid *Epilobium angustifolium* which, in spite of the changes in floral morphology and phenology, did not present pollinator shift, but have higher pollinator fidelity than diploids (Husband & Sabara 2003). Another case is the orchid *Gymnadenia conopsea*, in which tetraploid plants differed from diploids in floral display, scent, and color, but was visited by the same pollinator taxa, though, in a more effective way (Gross & Schiestl 2015). These studies suggest that although a shift in pollinator taxa is a remarkable potential effect of polyploidy, it is not the only consequence of polyploidy for plant pollination. Alterations in pollinator behavior, or the flower morphology, causing differences in pollen deposition on the pollinator's body may also significantly change the outcome of the pollination process (Borg-Karlson 1990). For example, among sympatric *Burmeistera* species sharing bats as their primary pollinators, it is the degree of anther exertion that determines the site of pollen deposition on the bat's head, avoiding the interspecific pollen transfer and the hybrid formation (Muchhala & Potts 2007).

Is it possible to make generalizations?

To answer this question, first, it is important to know how frequently pollinator shift is a result of hybridization and/or polyploidy. Based on the results of the literature survey, the pollinator shift was detected in 75 % of the cases involving hybridization, even when considering just the homoploids (nine shifts among the 12 homoploids taxa, 75 %) or the allopolyploids (12 shifts among the 16 taxa, 75 %). However, among allopolyploids, the genus *Nicotiana* alone responds for 68.75 % of the sampling and all of them presented pollinator shift. When removing *Nicotiana* allopolyploids from the survey, the frequency of pollinator shift among allopolyploids decreases to 20 % and considering just one *Nicotiana* allopolyploid, the rate will be 33 %. In both cases, the pollinator shift rate among allopolyploids is much lower than the one reported to



homoploids (75 %), but still much higher than reported to autopolyploids (11 %). Moreover, considering the importance of flower traits in mediating plant-pollinator interactions and the high frequency by which these traits are affected by hybridization and polyploidy (see also the synthesis in Tab. 1 and the discussion in the previous sections), we can predict a frequent effect of hybridization and polyploidy on plant-pollinator interactions.

The establishment of a strong reproductive barrier between cytotypes seems to rely on the balance between genomic differences and pollination barriers. Considering hybrids, the higher the genomic similarity between parentals (as homoploids, that does not depend on polyploidization to recovered fertility), the bigger the dependency on pollinator shift as a reproductive barrier (75 % of homoploids presented pollinator shift). In the other hand, the autopolyploids seems to present a lower dependency on pollinator shift as the main reproductive barrier (detected on 11 % of the taxa), with pre-pollination barriers, as morphology and phenology, along with the triploid sterility (post-pollination barrier), ensuring the reproductive barrier without the pollinator shift (e.g., see Pegoraro 2016). Nevertheless, in this prediction, it is worth noticing that autopolyploidy studies could be underestimated because of the sampling usually has a bias favoring allopolyploids (Barker *et al.* 2016; Ma *et al.* 2016).

In constructing such predictive scenarios, we also need to consider the specificity in the *continuum* of generalization-specialization in plant-pollinator interactions (Padyšáková *et al.* 2013; Brosi 2016; Carstensen *et al.* 2018). It is suggested that pollinator shift in hybrid plants, regardless of ploidy, should be expected for those plants that interact in a specialized way with their pollinators (Chase *et al.* 2010). In these systems, a small change in floral traits, such as flower scent, would have a great potential to be perceived by pollinators that might not visit the flower. This was reported in the hybridization zone of two *Yucca* species that develop a specialized interaction with their pollinating yucca moth: although progenitor plants produce different floral scent with no effect on pollinators, small changes in flower morphology between hybrids and parental plants may be important in limiting the introgression and ensuring a pre-zygotic reproductive barrier (Pellmyr 2003; Svensson *et al.* 2016).

Considering the interaction frequency, Vamosi *et al.* (2007) suggested that floral constancy for polyploids favors them to overcome the minority disadvantage since it results in a higher frequency of crossing between polyploids. Thus, it is expected a frequent interaction of such polyploid plants with pollinators of greater floral constancy, like social bees. These pollinators would favor the establishment of plants in the population in which polyploids are less abundant than diploids. However, the question remains whether this could be different for specialized and generalized plant-pollinator interactions.

To solve this question and as a future path, it is important to search for suitable plants to investigate how pollinator may be affected by plant hybridization and polyploidy. Although we did not emphasize generalization at first, the investigation using suitable model plants could contribute to the development of a deep mechanistic understanding of the ecological effects associated with the hybridization and polyploidy, which may then be used to build predictions to other systems. For this, we highlighted some Neotropical candidates for model plants, such as the genus *Nicotiana*, with many known polyploid and homoploid hybrids and specialized pollination systems, and some groups in the Orchidaceae family. Despite the low number of cases involving orchids in our review (four species and four hybrid entities; Tab. 1), this group represents a potential source of model systems based on its high incidence of hybrids (both allopolyploids and homoploids), their specialized plant-pollinator interactions, but also its frequent pollination by deceit, and diversity of reproductive systems (Jersáková *et al.* 2006; Cortis *et al.* 2009; Micheneau *et al.* 2009; Schiestl & Schlüter 2009; Scopece *et al.* 2010; Gross & Schiestl 2015; Johnson 2018). In the tropics, there are plenty of studies about orchid hybridization (Pinheiro *et al.* 2010; Moraes *et al.* 2013; Pinheiro & Cozzolino 2013; Vega *et al.* 2013; Pinheiro *et al.* 2015), polyploidization (Marques *et al.* 2014), and pollination (Singer 2003; Borba *et al.* 2011; Moré *et al.* 2012). However, despite the available information about the ecology and hybridization of these plants, the connections among hybridization-polyploidy-pollinators are still missing.

Conclusions

Intriguing questions emerge when considering whether and how floral change caused by hybridization and polyploidy could lead to changes in plant-pollinator interactions. Here we showed that both events of plant genome change often lead to pollinator shift, especially among hybrids taxa, which seems to be closely linked to changes in several floral traits. In fact, by modifying the floral traits related to plant-pollinator interactions, hybridization and polyploidy could impact the whole pollination process. In an evolutionary framework, future research may explore how rapid floral change caused by hybridization and polyploidy can lead to a new plant-pollinator interaction and evolutionary path. As a complementary step, we propose the investigation of the ecological bases of the pollination process in this scenario, so it would be possible to infer about its predictive value and generalization. In all cases, the pollinator-focused approach complements studies already done on how plant hybridization and polyploidy affect floral traits. This is certainly a frontier theme with great potential to foster future research that integrates genetics, ecology, and evolution in the study of plant-pollinator interactions.



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