

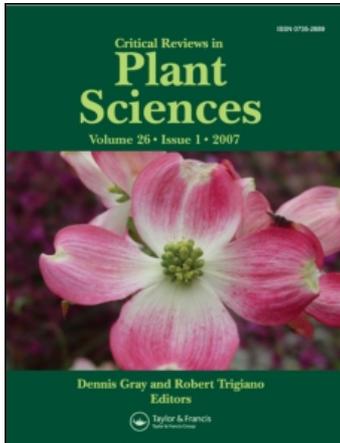
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Interspecific Pollen Transfer: Magnitude, Prevalence and Consequences for Plant Fitness

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Interspecific pollen transfer (IPT) is one of the mechanisms underlying potential competition among plants for pollinators, and it refers to movement of pollen between different plant species by pollinators that visit their flowers simultaneously. Two components of IPT, related to each other, are distinguished: (a) heterospecific pollen deposition (HPD) on conspecific stigmas, which may interfere with fertilization by conspecific pollen; and (b) conspecific pollen loss (CPL) on heterospecific flowers, which may reduce the amount of pollen transferred between conspecific flowers. Thus, IPT may lead to reciprocal losses for male and female functions of the plant, with potentially important ecological and evolutionary consequences. In this review, we explore the magnitude and prevalence of IPT, examining documented mechanisms and evaluating such potential ecological and evolutionary consequences. We compiled existing evidence of interspecific pollinator sharing and interspecific pollinator switching between flowers of different species in natural communities. We evaluated the relative importance of both HPD and CPL from studies comparing these variables in pure vs. mixed floral neighborhoods, analyzing evidence for the claim that IPT is an evolutionary force promoting character displacement in habitat affinity, flowering times, and floral morphology. We also examined the findings of hand-pollination experiments carried out to reveal different mechanisms by which heterospecific pollen can affect performance of native pollen. Finally, we review evidence for impacts of alien plant species on native species' reproduction, and briefly comment on risks of crop-to-wild gene flow imposed by the release of genetically modified (transgenic) crops through IPT.

Keywords conspecific pollen loss, heterospecific pollen deposition, hybridization, invasive flowering plants pollination, pollinator sharing, transgenic crops

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I. INTRODUCTION

Sympatric plant species may compete for space, resources, and also for services provided by mutualistic animals, such as pollination and seed dispersal, that can be crucial for their reproduction and population maintenance. Pollinator-mediated competition, specifically, occurs when at least one of the co-occurring plant species suffers decreased reproduction as a result of sharing pollinators (Waser, 1978a). There are at least two identified mechanisms that can lead to such competition: (1) "pollinator preference," also known as competition for visits (Campbell and Motten, 1985); it occurs when one plant usurps pollinators away from the other, reducing the pollinator visitation to the least preferred species, and (2) interspecific pollen transfer (hereafter IPT); it occurs when pollinators pick up pollen from one plant species and deposit them on another species as a consequence of mixed visits. This second mechanism, in turn, has two subcomponents: (a) heterospecific pollen deposition (hereafter HPD) on conspecific stigma, which may interfere with fertilization by conspecific pollen, and (b) conspecific pollen loss (hereafter CPL) on heterospecific flowers, which may reduce the amount of pollen transferred between conspecific flowers. Thus, from the viewpoint of each interacting species, IPT may lead to losses for male and/or female functions with potentially important ecological and evolutionary consequences (Waser, 1978a).

Most studies examining IPT among plants have focused on the first of such components, i.e., on HPD. In particular, a wide variety of laboratory experiments have aimed at elucidating physiological mechanisms through which heterospecific pollen may interfere with the fertilization of ovules by conspecific pollen (Kohn and Waser, 1985; Waser and Fugate, 1986; Harder *et al.*, 1992; Caruso and Alfaro, 2000). By contrast, CPL has been much less explored, despite being expected to occur at least at a similar frequency and to potentially have detrimental consequences on plant reproduction as well. CPL occurs when a

pollinator visiting a mixture of flowering species during a single foraging bout loses pollen grains of one species while visiting the other, thus decreasing the donor's chance to sire seeds (male fitness) and recipient's chance to set seeds (female fitness). In this sense, CPL is a special case of pollen discounting, since it reduces the amount of pollen available to conspecifics (Lloyd, 1992).

Competition for pollination among flowering plants could be a major force promoting evolutionary divergence in characteristics related to pollination, like habitat affinity, flowering time, floral morphology or pollinator identity, as was first proposed in the 19th century by Robertson (1895). However, discussion on mechanisms by which pollinator sharing may lead to competition was initiated many decades later by Levin and Anderson (1970). Since the evolutionary outcomes of interspecific interactions between plants via pollination are strongly mechanism-dependent (Rathcke, 1983), knowledge of the mechanism, and the magnitude of its impact is necessary in order to identify plausible ecological and evolutionary responses of potentially competing species (Galen and Gregory, 1989). The aim of testing this general hypothesis led to a broad range of studies of competition for pollination among co-flowering species and its relationship with habitat affinities (Kephart, 1983; Armbruster *et al.*, 1994), flowering times (Waser, 1978b; Campbell, 1985a; Campbell and Motten, 1985; Murray *et al.*, 1987; McGuire and Armbruster, 1991; Aizen and Vázquez, 2006), and floral morphology (Armbruster *et al.*, 1994; Murcia and Feinsinger, 1996).

More recently, a renewed interest on the impact of IPT has arisen as a consequence of introduction of alien flowering plant species and the release of genetically modified (transgenic) crops. Alien flowering plants might disrupt the pollination of natives (reviewed in Traveset and Richardson, 2006, and Bjerknes *et al.*, 2007), and transgenes might be transferred from genetically modified crops to wild relatives (reviewed in Snow *et al.*, 2005). Both phenomena can impose a serious threat to native species via IPT.

The main goal of this review is to explore the magnitude, prevalence, mechanisms and consequences of IPT in nature. To accomplish it, we compile existing evidence of interspecific pollinator sharing and interspecific pollinator switching between flowers of different species in natural communities and their consequences for co-flowering species. We evaluate the magnitude of IPT and the relative importance of both HPD and CPL from studies comparing these variables in pure vs. mixed floral neighborhoods. We also examine the evidence provided by hand pollination experiments on the different mechanisms by which heterospecific pollen can affect the performance of native pollen. Once we establish the extent of IPT and the relative importance of CPL and HPD in nature, we further evaluate the existing support for the claim that IPT is an evolutionary force promoting character displacement in habitat affinity, flowering times, floral morphology, and self-pollination. Finally, we review the current evidence on impacts of alien plant species on native's

reproduction, and the risks imposed by transgenic hybridization through IPT.

II. CONDITIONS FOR INTERSPECIFIC POLLEN TRANSFER

In order for IPT to take place between a given pair of flowering plant species, a set of conditions should be simultaneously met (Fig. 1). First, species should occur in the same locality or area. Second, sympatric species should overlap—totally or partially—in flowering times. Third, co-flowering sympatric species should share one or more pollinator species. Fourth, shared pollinators of co-flowering sympatric species should switch between flowers of different species during single foraging bouts or flights. Finally, to achieve HPD, pollen of both species should be deposited in a similar part of the pollinator's body, while this is not a necessary condition for CPL. In this section we explore the magnitude and extent of interspecific pollinator sharing and interspecific pollinator switches between flowers of sympatric co-flowering species.

A. Interspecific Pollinator Sharing

To estimate the extent of pollinator sharing, we compiled studies that addressed pollinator-mediated interactions between plant species in mixed neighborhoods, either in natural or artificial flower arrays. Table 1 summarizes the results of 16 field or artificial garden studies, which provided a measure of pollinator sharing. Most studies reported whether or not the involved species shared their main pollinator (i.e., the more frequent

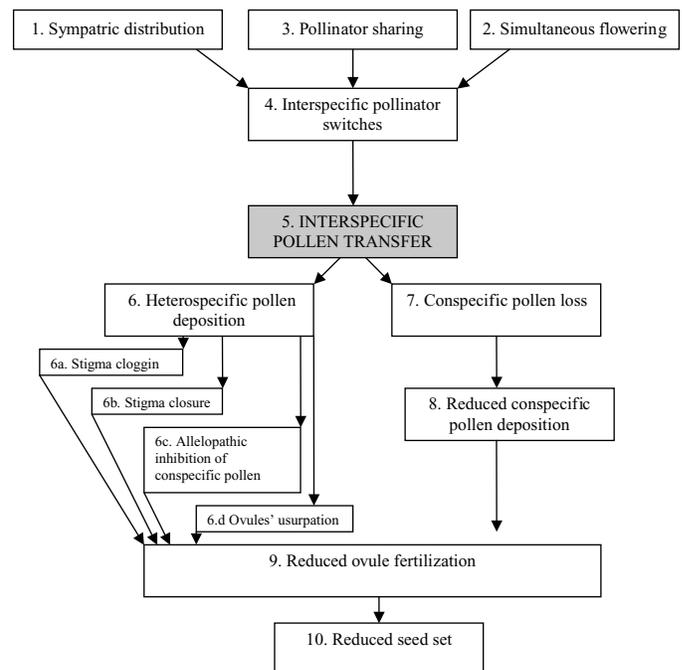


FIG. 1. Conceptual scheme representing the conditions for IPT, the involved mechanisms and reproductive consequences.

TABLE 1
Percent pollinator sharing and interspecific pollinator switches during visitor censuses between pairs of sympatric co-flowering plant species in natural populations and artificial floral neighborhoods

References	Species pair	Main pollinator	PS ¹	Interspecific pollinator switches ²
Armbruster and McGuire, 1991	<i>Aster sibiricus</i> ; <i>Erigeron glabellus</i> *	—	—	54% of FFT (only butterflies)
Bell <i>et al.</i> , 2005	<i>Mimulus ringens</i> ; <i>Lobelia siphilitica</i> *	Same	—	42% of PPT, 10% of FFT
Brown <i>et al.</i> , 2002	<i>Lythrum alatum</i> ; <i>Lythrum salicaria</i> †*	Same	—	33–65% of PPT
Campbell and Motten, 1985	<i>Stellaria pubera</i> ; <i>Claytonia virginica</i> *	Same	—	26 % (1981); 35% (1982) of FFT (from <i>C. virginica</i> to <i>S. pubera</i>)
Grabas and Laverty, 1999†	<i>Eupatorium maculatum</i> ; <i>Lythrum salicaria</i> †	Different	10–30%	
	<i>Eupatorium perfoliatum</i> ; <i>Lythrum salicaria</i> †	Different	5%	
	<i>Impatiens capensis</i> ; <i>Lythrum salicaria</i> †	Same	97%	
	<i>Viscaria vulgaris</i> ; <i>Melampyrum pratense</i>	Same	—	15% of PPT, 1.4% of FFT (only <i>B. hortorum</i>)
Kephart and Heiser, 1980	<i>Asclepias incarnata</i> ; <i>Asclepias verticillata</i> '*	—	55%	15.4–18.9% of PPT
	Larson <i>et al.</i> , 2006†	<i>Campanula rotundifolia</i> ; <i>Euphorbia esula</i> †	Different	36%
<i>Linum lewisii</i> ; <i>Euphorbia esula</i> †		Different	56%	
<i>Oxytropis lambertii</i> ; <i>Euphorbia esula</i> †		Different	66%	
<i>Lythrum alatum</i> ; <i>Lythrum salicaria</i> †		—	—	12 and 14% for bees and 39 and 13% for butterflies, <i>L. salicaria</i> and <i>L. alatum</i> respectively
McGuire Armbruster, 1991	<i>Saxifraga reflexa</i> ; <i>Saxifraga tricuspidata</i> *	Same	—	4.2% of FFT
Moragues and Traveset, 2005	<i>Anthyllis cytisoides</i> ; <i>Carpobrotus acinaciformis</i> †	Same	44%	
	<i>Cistus monspeliensis</i> ; <i>Carpobrotus acinaciformis</i> †	Same	71%	
	<i>Cistus salviifolius</i> ; <i>Carpobrotus edulis</i> †	Same	80%	
	<i>Lotus cytisoides</i> ; <i>Carpobrotus edulis</i> †	Same	56%	
	<i>Orobanche elatior</i> ; <i>Centaurea scabiosa</i>	Same	41%	<12% of PPT
Schemske, 1981	<i>Costus allenii</i> ; <i>Costus laevis</i>	Same	100%	Mixed visits in 50% (>2 flowers) and 100% (>7 flowers) of foraging bouts
Totland <i>et al.</i> , 2006	<i>Melampyrum pratense</i> ; <i>Phacelia thanacetifolia</i> †*	Same	—	Mixed visits in 24% (disturbed) and 29% (undisturbed) of censuses
Jakobsson <i>et al.</i> , 2008	<i>Carpobrotus spp</i> †; <i>Dorycnium hirsutum</i>	—	—	7.4% of arrivals to <i>D. hisutum</i>
	<i>Carpobrotus spp</i> †; <i>Asphodellus aestivus</i>	—	—	8.5% of arrivals to <i>A. aestivus</i> flowers
	<i>Carpobrotus spp</i> †; <i>Helichrysum stoechas</i>	—	—	2.5% of arrivals to <i>H. stoechas</i> flowers
	Waser, 1978b	<i>Delphinium nelsoni</i> ; <i>Ipomopsis aggregata</i> *	Same	—

¹PS = percent similarity in pollinators composition

²FFT = total flower to flower switches during the visitors census, PPT = total plant to plant switches during the visitors census

† Alien plant species

*Experimental artificial mixed neighborhood achieved by adding potted plants or flowers of either one or both species.

visitor species), whereas fewer provided a quantitative measure of percent pollinator sharing (PS; range = 0–100%), which takes into account not only the number of species shared but also the relative importance of each visitor to each plant species. A PS = 100% indicates complete overlap of pollinator groups while a PS = 0% indicates nil overlap in pollinator groups between the two plants. In studies that did not report PS values (e.g., Campbell and Motten, 1985; Larson *et al.*, 2006), but provided the input data (i.e., the abundance or visitation frequency of each flower visitor to each plant species), we estimated PS values following Hansen (2000).

PS ranged from 5 to 100% (median = 55.5%) and, as expected, species pairs sharing their main pollinator had relatively high PS values (median = 71%, range = 41–100%; Table 1). It is interesting, however, that even some species pairs whose main pollinators are different, had mid to high PS values (median = 45.5%, range = 36–66%), suggesting that secondary pollinators might also contribute to IPT (e.g., Jakobsson *et al.*, 2008).

It is important to note that both identity of the main pollinator (same or different) and PS refer to pollinator species, not necessarily implying thus that the same individuals visit both plant species. Even though plant species may present considerable similarity with respect to their pollinator faunas, resource partitioning might exist if individual pollinators show a high constancy or preference to flowers of a given species (Kephart, 1983; Goulson, 1994). Pollinator constancy, the tendency of individual pollinators to forage within single species in the presence of other suitable species (Levin and Anderson, 1970), reduces the extent to which reproductive interference is possible through interspecific pollination. Pollinator constancy, also known as insect flower constancy (Goulson, 1994) depends on several variables, including the insect's ability to discriminate between different plant species and the time it takes this discrimination to occur, the relative abundance and spatial arrangement of the plant species involved, and any difference in attractiveness that might result in the preference for one species over another (Kephart, 1983).

B. Pollinator Switches between Flowers of Different Species

Pollinators switching between different flowering species are good candidates to act as pollen transfer agents. The most common and accurate measures of interspecific pollinator switches are the percent of plant-to-plant transitions (PPT) or flower-to-flower transitions (FFT) that are interspecific. Nevertheless, some studies also provide the percent of censuses with at least one mixed visit; that is, an interspecific pollinator switch or flight (Schemske, 1981; Totland *et al.*, 2006), or the percent of arrivals to one of the species that comes from the other species (Campbell and Motten, 1985; Jakobsson *et al.*, 2008) (Table 1).

Reported levels of interspecific pollinator switches in mixed neighborhoods ranged between 9 and 65% of PPT

(median = 18.9%), and between 1.4 and 54% of FFT (median = 12%) (Table 1). The difference between both measures might reflect the trend of pollinators to visit many flowers within each plant before moving to the next individual. Thus, plants with greater flower display are expected to experience less interspecific pollinator switches, and, if visitors effectively transfer pollen, higher levels of geitonogamy than plants with smaller flower displays.

The magnitude of interspecific pollinator switches between a given pair of plant species can vary in time (e.g., between years; Campbell and Motten, 1985) and in space (e.g., between habitat types; Totland *et al.*, 2006). Moreover, different pollinator species have been found to exhibit different levels of constancy to flowers (Levin and Anderson, 1970). Hummingbirds are usually reported to be indiscriminate in their visitation (Rathcke, 1983; and references therein). On the contrary, bees in general and bumblebees in particular, usually exhibit high levels of pollinator constancy (Goulson, 1994). This is supported by the finding that the lowest percent of interspecific FFT corresponded to *Bombus hortorum* (Table 1, Jennersten and Kwak, 1991). Likewise, Rathcke (1988) found that in spite of high bumblebee pollinator sharing between four co-flowering shrubs that grew intermingled and were visited by generalist bees, only three (2%) out of 180 bumblebees marked over four years were recaptured on flowers of another species. Thus, the identity of pollinator species might affect the magnitude of IPT due to its influence on pollinator constancy.

The importance of pollinator identity on interspecific pollinator switching, and in turn, on IPT was exemplified by the study of Jakobsson *et al.* (2008). In this study, amounts of pollen carried by pollinators departing from the invasive *Carpobrotus* sp. and amounts of pollen carried by pollinators arriving to the target species *Dorycnium hirsutum* were compared among major pollinators. Despite the fact that *Rhodanthidium septemdentatum* and *Apis mellifera* picked up similar amounts of *Carpobrotus* pollen, *R. septemdentatum* individuals arriving to *D. hirsutum* flowers carried significantly more *Carpobrotus* pollen than *A. mellifera*, because the former was more probable to switch directly from *Carpobrotus* to *D. hirsutum* flowers than the latter (Jakobsson *et al.*, 2008). Thus, the identity of shared pollinators may influence the level of IPT, and probably a minor fraction of a plant's pollinator species assemblage accounts for substantial levels of IPT through switching between different flowering species.

III. INTERSPECIFIC POLLEN TRANSFER

A. Magnitude of Conspecific Pollen Loss and Heterospecific Pollen Deposition in Nature

Many studies present anecdotal evidence of heterospecific pollen grains on stigmas of other species in natural communities (e.g., Aizen and Vázquez, 2006). Nevertheless, if the magnitude of such IPT is consistently low, no impact on plants' fitness and, in turn, in plants' evolution should be expected. Therefore, in

order to quantify the magnitude of CPL and HPD and the influence of the floral neighborhood, comparisons of natural levels of conspecific and heterospecific pollen deposition in stigmas of focal plants growing in the presence (mixed neighborhood) vs. the absence (pure neighborhood) of a second co-flowering species are necessary. In Table 2 we compiled information from 13 independent studies, assessing the effect of the floral neighborhood on the amount of conspecific and heterospecific pollen deposition on stigmas of focal plant.

Studies differed in the identity of the species pair, habitat type, spatial scale of the neighborhood, relative densities of flowering species, and experimental approach. Neighborhood comparisons were obtained by different means: (1) experimental plots of potted plants (e.g., Waser, 1978b; McGuire and Armbruster, 1991; Bell *et al.*, 2005), (2) removal of co-flowering species (e.g., Caruso, 1999; Murphy and Aarsen, 1995), or (3) comparison of natural contrasting densities of co-flowering species (e.g., Ghazoul, 2002, 2004; Caruso, 2000; Moragues and Traveset, 2005).

The presence of co-flowering species had a significant effect, either negative (35%) or positive (10%) on conspecific pollen deposition in 45% of a total of 31 comparisons (Table 2). In at least one study there is clear evidence that CPL is entirely responsible for the reduced conspecific pollen deposition (Feinsinger *et al.*, 1991), while in the remaining studies, either CPL or competition for visits (Caruso, 1999, 2000), or a combination of both (Ghazoul, 2002, 2004; Bell *et al.*, 2005) are pointed as potential mechanisms mediating the negative effect of heterospecific flowers on conspecific pollen deposition. Not surprisingly, in the only study where the presence of co-flowering species consistently increased the deposition of conspecific pollen, the focal plant was a rewardless orchid (*Anacamptis morio*), whose flowers lack rewards (pollen or nectar) for visiting insects, and therefore benefits from sharing pollinators with rewarding neighbors (Johnson *et al.*, 2003). On the other hand, only in 4 (11%) out of 36 cases, HPD increased significantly in mixed compared to pure neighborhoods (Table 2). It is worth noting that in two out of four cases, species experienced increased HPD in only one of three runs or trials (Feinsinger *et al.*, 1986), suggesting that the outcome of this interspecific interaction might vary even at short time scales. In addition, in one of the two remaining cases of increased HPD, the species involved were wind-pollinated grasses (Murphy and Aarsen, 1995), whose pollen release is expected to be more profuse and the pollen vector less specific than in animal-pollinated plants.

Overall, there seems to be a moderate influence of floral neighborhood on IPT, and available data so far indicate that regardless its consequences on reproduction, the reduction in conspecific pollen deposition (probably as a consequence of increased CPL) is a more common outcome of pollinator sharing than HPD, at least in animal-pollinated plants. Nevertheless, it is worth noting that the more prevalent reduction of conspecific pollen deposition in mixed neighborhoods may confound the effect of CPL with that of reduced visitation due to competition

for visits (see above). Alternatively, assuming that reduced conspecific pollen deposition reliably reflects CPL (e.g., Feinsinger *et al.*, 1991), a lower percent of HPD than CPL might be due to a significant fraction of the heterospecific pollen being deposited on flower structures of the co-flowering species other than stigmas (Murcia and Feinsinger, 1996).

On the other hand, 55% of the studies failed to find a significant influence of floral neighborhood on HPD and CPL (Table 2). The amounts of pollen transferred to stigmas depend on the frequency of pollinator switching, the amount of heterospecific pollen adhering to switching pollinators, and the number of pollen grains actually transferred to stigmas of focal species during each visit (Jakobsson *et al.*, 2008), which in turn depends on pollinator constancy and the placement of pollen on pollinators' body. High pollinator constancy, for instance, is probably responsible for the low IPT found between *Centaurea scabiosa* and its holoparasite *Orobancha elatior* in the British grasslands (Ollerton *et al.*, 2007). These two species partially overlap in flowering phenology and pollinator sharing; *Bombus lapidarius* is the only pollinator of the parasite, besides being the second most frequent and efficient pollinator of the host. Despite obligate sympatry of the parasite with the host at a short spatial scale, pollinators tend to visit flower sequences from the same species during single foraging bouts (Ollerton *et al.*, 2007).

However, low pollinator constancy does not necessarily imply a higher IPT. Despite obligate pollinator inconstancy in the tropical nectarless species *Cassia reticulata* and *Cochlospermum vitifolium*, whose pollinators must obtain nectar elsewhere, Snow and Roubik (1987) found that IPT was extremely rare (<1% of *C. vitifolium* stigmas exposed to a single visit, received one or two foreign pollen grains). This suggests, thus, that mechanisms other than pollinator constancy (e.g., differentiated pollen deposition on the pollinator's body) might be responsible for low IPT values.

The magnitude of IPT is expected to be sensitive to the density and intermixing of species (Rathcke, 1983) and might be substantial in small spatial scales when pollinators show high inconstancy. For instance, Brown and Mitchell (2001) used fluorescent dye particles as pollen analogous to track pollen transfer in mixed arrays of potted flowering plants of the invasive *Lythrum salicaria* and the native *L. alatum*, finding that after only three hours of exposure to pollinators, >63% of the native flowers received small amounts of particles from the invasive congener, what suggested a high rate of HPD. Therefore, differences in the levels of HPD and CPL among studies are likely to reflect the inter-study variation in the experimental spatial scale and degree of intermingling.

Despite IPT and changes in visitation frequency are two different outcomes of interactions between co-flowering species via pollination, levels of IPT may vary with changes in visitation rates by shared pollinators. Through a manipulative field experiment, Lopezaraiza-Mikel *et al.* (2007) investigated the impact of alien *Impatiens glandulifera* on an entire community of co-flowering native plants. Despite overall higher flower

TABLE 2

Effect of the presence of a co-flowering species on conspecific and heterospecific pollen deposition on stigmas of focal species (comparison between mixed vs. pure neighborhoods).

References	Co-flowering species	Focal species	Other variables	Conspecific pollen ¹	Heterospecific pollen
Bell <i>et al.</i> , 2005	<i>Lobelia siphilitica</i>	<i>Mimulus ringens</i>	—	(-)	NS (+)
Kephart and Heiser, 1980	<i>Asclepias verticillata</i>	<i>Asclepias incarnata</i>	1977, 1978		NS(+), NS (+)
Waser, 1978b	<i>Asclepias incarnata</i>	<i>Asclepias verticillata</i>	1977, 1978		NS(+), NS(+)
	<i>Ipomopsis aggregata</i>	<i>Delphinium nelsoni</i>	—		? (+)
Feinsinger <i>et al.</i> , 1991	<i>Delphinium nelsoni</i>	<i>Ipomopsis aggregata</i>	—		? (+)
	<i>Cephaelis elata</i>	<i>Belseria triflora</i>	—	NS (-)	
Moragues and Traveset, 2005	<i>Carpobrotus edulis</i>	<i>Palicourea lasiorrachis</i>	—	(-)	
		<i>Cistus salviifolius</i>	—		? (+)
Ghazoul, 2002, 2004	<i>Chromolaena odorata</i>	<i>Lotus cytisoides</i>	—		NS(-)
		<i>Dipterocarpus obtusifolius</i>	—	(-)	(+)
Grabas and Laverty, 1999	<i>Lythrum salicaria</i>	<i>Eupatorium maculatum</i>	Swampy oxbow, low meadow		NS, NS(+)
	<i>Lythrum salicaria</i>	<i>Impatiens capensis</i>	Swampy oxbow, low meadow	NS(-), NS(+)	NS, NS
Caruso, 1999, 2000	<i>Castilleja linariaefolia</i>	<i>Ipomopsis aggregata</i>	Artificial Garden, natural populations	(-), (-)	NS(+)
Johnson <i>et al.</i> , 2003	<i>Allium schoenoprasum</i>	<i>Anacamptis morio</i>	—	(+)	
	<i>Geum rivale</i>	<i>Anacamptis morio</i>	—	(+)	
Larson <i>et al.</i> , 2006	<i>Euphorbia esula</i>	<i>Calylophus serrulatus</i>	—	(-)	NS
	<i>Euphorbia esula</i>	<i>Campanula rotundifolia</i>	—	(-)	NS(+)
	<i>Euphorbia esula</i>	<i>Linum lewisii</i>	2000, 2001	(-), (-)	NS(+), NS(+)
	<i>Euphorbia esula</i>	<i>Oxytropis lambertii</i>	2000, 2001	NS(+), (-)	NS, NS(-)
	<i>Euphorbia esula</i>	<i>Sphaeralcea coccinea</i>	2000	(-)	NS(+)
Feinsinger <i>et al.</i> , 1986	Various	<i>Vicia americana</i>	2001	NS(+)	NS
		<i>Besleria triflora</i>	Run 1, 2, 3	NS(+), NS(-), NS(-)	NS(-), NS(+), NS(-)
		<i>Drymonia rubra</i>	Run 1, 2, 3	NS(-), (+), NS(+)	NS(-), NS(+), NS(+)
		<i>Hansteinia blepharorachis</i>	Run 1, 2, 3	NS, NS(-), NS(+)	NS(-), NS(-), (+)
		<i>Raziceas spicata</i>	Run 1, 2, 3	NS(+), NS(-), NS(+)	(+), NS(-), NS(-)
Murphy and Aarsen, 1995	<i>Phleum pratense</i> †	<i>Elytrigia repens</i> †	-	NS(-)	(+)

Symbols: (-) = significantly negative effect of co-flowering species; (+) = significantly positive effect of co-flowering species; NS = non significant effect of co-flowering species; (+) = non significant positive trend; (-) = non significant negative trend; ? = statistical significance not evaluated between treatments. † wind pollinated grasses

visitation in invaded plots, the pollen transport networks were dominated by alien pollen grains, suggesting that the positive effect of alien flowers on native flower visitation might be offset by the negative effect of IPT. Likewise, Larson *et al.* (2006) found that in prairies invaded by *Euphorbia esula*, flowers visitation rate was uncorrelated with the amount of conspecific pollen in

stigmas, suggesting that the enhanced visitation was counteracted by a loss of conspecific pollen in heterospecific flowers. Thus, in mixed neighborhoods with high levels of interspecific pollinator switching, higher visitation rates may not translate into increased facilitation for pollination, due to a parallel increase either in HPD, CPL, or both.

In short, there seems to be a low to moderate influence of floral neighborhood on HPD and CPL respectively, and the magnitude of IPT may vary with pollinator constancy, placement of pollen on pollinator's body, and spatial scale. In most cases, the impact of increased HPD or CPL on plant reproduction is unknown. Nevertheless, there is some evidence that within the range of IPT experienced in natural systems, the impact of CPL-mediated reduced conspecific pollen deposition on plant reproduction might be more deleterious than that of HPD, as will be illustrated in the next section.

B. The Relative Impact of CPL and HPD on Plant Reproduction

1. Progeny Quantity

Interspecific pollen transfer may reduce female and male success, through HPD or CPL (Fig. 1). To date, all studies have measured the impact of either HPD or CPL on female success, more specifically, on seed output. After comparing the magnitude and extent of HPD and CPL in mixed neighborhoods, a key question is whether these mechanisms have a measurable impact on plant reproduction. The idea that CPL might be more detrimental than HPD for plant reproduction has received support from both direct and indirect evidences. Two sets of studies carried out in the mesic lowland deciduous forests of North America (Campbell, 1985b; Campbell and Motten, 1985) and in the tropical cloud forests of Costa Rica (Feinsinger and Tiebout, 1991; Feinsinger *et al.*, 1991; Murcia and Feinsinger, 1996), respectively, provided the most detailed evaluation of both the magnitude and consequences of CPL and HPD for plant reproduction.

Study Case I: Mesic lowland deciduous forests of North America. *Claytonia virginica* and *Stellaria pubera* partially overlap in blooming time. Through a set of field experiments, Campbell (1985b) found that the presence of *C. virginica* flowers decreased seed set in *S. pubera*. In a related paper, Campbell and Motten (1985) investigated the potential mechanisms (pollinator preference, or IPT) by measuring the effect of *C. virginica* both on the quantity and quality of pollinator visits. They found that visitation frequency to *S. pubera* was unaffected by the presence of heterospecific flowers, that interspecific pollinator switches did not result in substantial HPD, and that seed set was unaffected by hand pollination with heterospecific pollen. These evidences indicated that neither pollinator preference nor HPD were responsible for competitive effects and for the reduced seed set in *S. pubera* in the presence of *C. virginica*. Next, Campbell and Motten (1985) investigated the role of the previous history of visits arriving to *S. pubera* on their stigmatic pollen loads, finding that similar amounts of conspecific pollen were deposited after a single visit, regardless of whether the pollinator arrived from *C. virginica* or a pistillate *S. pubera* (neither of them are sources of conspecific pollen). Pollen loads were higher, however, when visits arrived from staminate *S. pubera* flowers. Thus, CPL showed to be the primary mechanism of competition for

pollination between the two co-flowering species (Campbell and Motten, 1985).

Study Case II: Costa Rican cloud forest. *Cephalis elata*, *Palicourea lasiorrachis*, and *Besleria triflora* are pollinated nearly 100% by a single hummingbird species (*Lampornis calolaema*), and because of similar placement of their reproductive parts, all three species deposit pollen on, and pick pollen from, similar parts on *Lampornis* bill. Feinsinger *et al.* (1991) found that experimental enriching of floral neighborhood with heterospecific flowers of *C. elata* decreased both pollination levels and seed set in the self-incompatible *P. lasiorrachis* and the self-compatible *B. triflora*. Since visitation frequency remained unaltered, CPL was proposed as a possible explanation for reduced pollination. Furthermore, as higher levels of *C. elata* pollen did not seem to impair fertilization of focal plant species, the reduced seed set in the presence of *C. elata* flowers seems to be a direct consequence of CPL.

This mechanism was later confirmed by a laboratory experiment that evaluated the effect of intervening visits to *C. elata* on intraspecific pollen transfer between flowers of *B. triflora* or of *P. lasiorrachis* by captive *Lampornis* (Feinsinger and Tiebout, 1991). The few *C. elata* grains deposited did not appear to restrict the space available for conspecific pollen in either *B. triflora* or *P. lasiorrachis*, but the previous visit to *C. elata* flowers led to decreased number of conspecific pollen grains and pollen tubes in their stigmas. This result was consistent with the detrimental effects of *C. elata* flower density on pollination and seed set found in the field (Feinsinger *et al.*, 1991), confirming that CPL was the underlying mechanism for the reduced reproductive success in both species.

Using a similar experimental approach, Murcia and Feinsinger (1996) found that in a guild of six hummingbird-pollinated plants, CPL reduced by 76% the pollen transferred among conspecific flowers. Moreover, the amount of *Palicourea* pollen on anthers and stigmas of heterospecific flowers was smaller than those that disappeared from bird bills, suggesting that missing pollen must have been scraped off on floral structures others than stigmas. This finding further implies that the estimating CPL from the amount of pollen deposited on heterospecific stigmas may underestimate the actual levels of pollen wastage due to IPT.

Although more studies of this kind are necessary before a generalization can be made, the most detailed field and laboratory experiments to date provide evidence that CPL might negatively affect maternal fitness by decreasing the availability of pollen to sire ovules, and in turn, the number of seeds set. Furthermore, IPT might also affect another component of fitness, that is, the quality of seeds.

2. Progeny Quality

IPT might affect not only the quantity but also the quality of the progeny, due to its influence on outcrossing rates, which was predicted by Campbell (1985a; see also Feinsinger *et al.*, 1991). This prediction was confirmed by Bell *et al.* (2005), who found

that competition for pollination with *Lobelia siphilitica* dramatically lowered both seed set and outcrossing rate in *Mimulus ringens*. Reduced pollinator visitation together with CPL partially explained seed set reduction. In addition, CPL is the most likely cause of reduction in outcrossing rate. This influence has important implications for plant fitness since reproduction depends upon both the quality and quantity of offspring (Bell *et al.*, 2005). In species exhibiting inbreeding depression, which reflects in a reduced mean fitness of self-progeny in comparison to outcrossed progeny, the overall effect of competition for pollination suggests that the reduction in maternal fitness might be even more pronounced than that estimated when examining only seed set, because inbreeding depression might express at any stage of the progeny life cycle (Husband and Gurney, 1998).

Finally, high incidence of HPD in nature might not necessarily impair reproduction if heterospecific pollen does not alter conspecific pollen performance. This was found, for instance, in natural populations of *Viscaria vulgaris*, where despite visitors depositing on average 18% of heterospecific pollen reproduction was not affected by the presence of heterospecific pollen, as corroborated by hand pollinations (Kwak and Jennersten 1991, see Table 3). Therefore, experimental testing of the potential of heterospecific pollen to impair reproduction is fundamental before HPD can be proposed as a possible cause of reproductive failure.

C. Impact of HPD on Plant Reproduction and Mechanisms Involved as Revealed by Hand Pollination Experiments

A number of mechanisms acting at different stages of the pollination and fertilization process have been proposed to explain how HPD can depress reproduction (Fig. 1). These are: “stigma clogging,” that is the physical obstruction or saturation of stigmatic surface by heterospecific pollen (Galen and Gregory, 1989), “stigma closure,” that is the loss of stigmatic receptivity (Waser and Fugate, 1986); “allelopathic inhibition of conspecific pollen germination or of pollen tube’s growth” (Sukhada and Jayachandra, 1980; Thomson *et al.*, 1981), “stylar clogging” (Brown and Mitchell, 2001), or “usurpation of ovules” by heterospecific pollen tubes arriving prior to conspecific pollen tubes (Harder, 1992).

One way that has proven fruitful to test these potential alternative mechanisms is the use of hand pollination experiments. Even though hand pollinations do not provide an estimate of the importance of HPD on plant reproduction in nature (Galen and Gregory, 1989), controlled experiments have the advantage of separating the effect of the presence of heterospecific pollen on stigmas from any other potentially co-varying factor, allowing the simultaneous evaluation of underlying mechanisms.

Table 3 summarizes the results of hand pollination experiments from 15 studies, evaluating the effect of heterospecific pollen applied under different experimental protocols on plant’s

reproduction. Most studies compared the effect of hand pollinations with pure conspecific pollen loads vs. mixes of conspecific and heterospecific pollen on seed set (Table 3). Some studies applied conspecific and heterospecific pollen simultaneously to achieve the mixed pollen treatment (e.g., Kwak and Jennersten, 1991; Brown and Mitchell, 2001; Larson *et al.*, 2006), whereas others applied heterospecific pollen prior to conspecific pollen in mixed treatments (e.g., Waser and Fugate, 1986; Caruso and Alfaro, 2000). Further, other studies compared the effect of heterospecific pollen application followed by free natural pollination vs. conspecific pure pollination (Kwak and Jennerstenn, 1986, 1991; Jakobsson *et al.*, 2008, data not shown). Since we think this latter comparison might confound the potential negative effect of heterospecific pollen with that of pollen limitation due to low natural visitation, these comparisons were not analyzed here. Finally, some studies compared hand pollination with heterospecific vs. conspecific pollen, which evaluate the chance of hybridization between congeners (Schemske, 1981; Harder *et al.*, 1992; Brown and Mitchell, 2001).

Many studies found detrimental effect of simultaneous application of heterospecific and conspecific pollen on seed set (Waser, 1978b; Brown and Mitchell, 2001; Moragues and Traveset, 2005; Larson *et al.*, 2006), while others found no detectable effect (Kohn and Waser, 1985; Armbruster and McGuire, 1991; Kwak and Jennersten, 1991; Caruso and Alfaro, 2000), which suggests that the impact may vary among the species pairs involved. Furthermore, the mechanism underlying the decline in reproduction through HPD appears to partly depend on the time of conspecific and heterospecific pollen arrival. This might also explain contrasting results found in experiments where heterospecific pollen was applied prior or simultaneously with conspecific pollen; heterospecific pollen applied prior to conspecific pollen had a more detrimental effect on seed set than simultaneous mixed pollination (e.g., Kohn and Waser, 1985; Waser and Fugate, 1986; Caruso and Alfaro, 2000; but see Kwak and Jennersten, 1991).

Prior application of heterospecific pollen might lead to stigma closure. For instance, mixed hand-pollinations (with con- and heterospecific pollen of *Delphinium nelsonii*) simultaneously applied to *I. aggregata* stigmas showed no decline in seed set (Kohn and Waser, 1985); however, when applied 6 h before, *D. nelsonii* pollen often caused stigma lobes of *I. aggregata* to close together within 1.5–6 h, reducing receptivity and, in turn, decreasing mean seed set by almost 50% (Waser and Fugate, 1986), a result consistent with those observed in previous experiments (Waser, 1978b, see Table 3). Alternatively, a similar study suggests that mechanisms other than stigma closure may mediate the negative effect of heterospecific pollen precedence on seed set. Caruso and Alfaro (2000) found that mixed simultaneous pollination with *Castilleja linariaefolia* reduced conspecific pollen deposition by 39% in *Ipomopsis aggregata* compared with conspecific pollination, but this reduction did not translate in fewer seeds per fruit. By contrast, previous application of heterospecific pollen reduced conspecific pollen deposition by 30% and

TABLE 3

Effect of different hand pollination treatments with conspecific and heterospecific pollen on conspecific pollen deposited, pollen germinated, and seedset of focal plant species.

Reference	Focal species	Source heterospecific pollen	Response Variable	Het+con vs. con ¹	het→con vs. con	Het vs. con	het→open vs. open
Campbell and Motten, 1985	<i>Stellaria pubera</i>	<i>Claytonia virginica</i>	Seedset		NS(+)		
Brown and Mitchell, 2001	<i>Lythrum alatum</i>	<i>Lythrum salicaria</i>	Seedset	(-) ²		(-)	
Larson <i>et al.</i> , 2005	<i>Linum lewisii</i>	<i>Euphorbia esula</i>	Seedset	(-)			
Moragues and Traveset, 2005	<i>Cistus monspeliensis</i>	<i>Carpobrotus</i>	Seedset	(-)			
	<i>Cistus salviifolius</i>	<i>Carpobrotus</i>	Seedset	(-)			
Kwak and Jennersten, 1986, 1991	<i>Viscaria vulgaris</i>	<i>Lupinus polyphyllus</i>	Seedset	NS(-)	NS(+)		
Kwak and Jennersten, 1991	<i>Viscaria vulgaris</i>	<i>Melampyrum pratense</i>	seed/ovules	NS(+)			
	<i>Melampyrum pratense</i>	<i>Viscaria vulgaris</i>	Seedset	NS(-)			
Caruso and Alfaro, 2000	<i>Castilleja linariaefolia</i>	<i>Ipomopsis aggregata</i>	Seedset	NS(-)	(-)		
Kohn and Waser, 1985	<i>Ipomopsis aggregata</i>	<i>Delphinium nelsoni</i>	Seedset	NS			
Waser, 1978b	<i>Delphinium nelsoni</i>	<i>Ipomopsis aggregata</i>	Seedset	NS(-)			
	<i>Ipomopsis aggregata</i>	<i>Delphinium nelsoni</i>	Seedset	(-)			
Waser and Fugate, 1986	<i>Ipomopsis aggregata</i>	<i>Delphinium nelsoni</i>	Seedset		(-)		
Harder <i>et al.</i> , 1992	<i>Erythronium albidum</i>	<i>Erythronium americanum</i>	Seedset	(-)		(-)	
	<i>Erythronium americanum</i>	<i>Erythronium albidum</i>	Seedset	NS		(-)	
Schemske 1981	<i>Costus allenii</i>	<i>Costus laevis</i>	Seedset			(-)	
	<i>Costus laevis</i>	<i>Costus allenii</i>	Seedset			(-)	
Armbruster and McGuire 1991	<i>Erigeron glabellus</i>	<i>Aster sibiricus</i>	Seedset	NS(+)			
	<i>Aster sibiricus</i>	<i>Erigeron glabellus</i>	Seedset	NS(-)			
Galen and Gregory 1989	<i>Polemonium viscosum</i>	<i>Castilleja occidentalis</i>	Pollen deposited				NS(-)
			Pollen germinated				(-)
			Seedset				(-)
	<i>Polemonium viscosum</i>	<i>Mertensia viridis</i>	Pollen deposited				NS(-)
			Pollen germinated				(-)
			Seedset				(-)
Jakobsson <i>et al.</i> , 2008	<i>Asphodelus aestivus</i>	<i>Carpobrotus</i> sp.	Fruit set	? (-)			
	<i>Dorycnium hirsutum</i>	<i>Carpobrotus</i> sp.	Seed set	? (-)			
	<i>Helichrysum stoechas</i>	<i>Carpobrotus</i> sp.	Seed set	? (-)			

¹Treatments abbreviations: con = pure conspecific hand pollination; het = pure heterospecific hand pollination; open = exposure to natural pollination ; het + con = mixed heterospecific and conspecific hand pollination; het → con = conspecific hand pollination after heterospecific hand pollination; het → open = exposure to natural pollination after heterospecific hand pollination.

²Significance as in Table 2.

seed set by 38%, regardless of stigma closure. In addition, only 12% of *I. aggregata* stigmas closed after pollination with *C. linariaefolia*, what suggests that other factors, like the adhesion, germination, tube growth or fertilization success may cause the negative effect of heterospecific pollen precedence (Caruso and Alfaro 2000).

On the other hand, other studies failed to find a negative effect of heterospecific pollen on reproduction, independently of the time of pollen application. For instance, Armbruster and McGuire (1991) found that the time of arrival of conspecific and heterospecific pollen on the stigmas of *Erigeron glabellus* and *Aster sibiricus* had a similar nil effect on female fecundity. Likewise, Kwak and Jennersten (1991) reported that *Viscaria vulgaris* was not affected by hand pollination with heterospecific pollen, independently of the time at which such pollen was applied (i.e., simultaneous mixed pollination vs. heterospecific pollination 6 hours prior to conspecific pollination). Thus, heterospecific pollen does not necessarily affect seed set due to stigma closure, stigma clogging or inhibition of tube growth, and in some species, this effect only arises when that heterospecific pollen arrives prior to conspecific pollen.

The mechanism by which heterospecific pollen appears to have the strongest effect on plant's reproduction is allelopathic inhibition, which may act at different stages of the pollination process. Thomson *et al.* (1981) analyzed the functional response of ovule development in emasculated flowers of the self-incompatible *Diervilla lonicera* to the proportion of foreign pollen from *Hieracium floribundum*. They detected a striking nonlinear decrease in ovule development with increasing proportions of heterospecific pollen, to the extent of complete reproductive failure at proportions of heterospecific pollen > 50%, and a decrease in ovule development detectable even at low proportions (5–10%). This strong depressive effect of moderate concentrations suggests that the simple passive mechanism of physical displacement of conspecific grains in a limited stigmatic surface provide insufficient explanation. The authors hypothesized that heterospecific pollen might inhibit germination or growth of conspecific pollen by active means of the type of incompatibility reactions (Thomson *et al.*, 1981). Active inhibition via allelopathic effects has been demonstrated for pollen of the weedy *Parthenium hysterophorus*; the pollen and pollen aqueous extraction of this weed inhibits not only seed set but also pollen germination and tube growth in pollen cultures in several species (Sukhada and Jayachandra, 1980). This mechanism is expected to be more important in nature than other proposed mechanisms (see above), given that a very small amount of heterospecific pollen might be enough to trigger allelopathic reactions, and subsequent reproduction failure (Murphy and Aarsen, 1995).

Finally, another important mechanism through which HPD may impair reproduction in closely taxonomically related species is the hybridization or usurpation of ovules by pollen tubes of heterospecific pollen (Harder *et al.*, 1992; Burgess *et al.*, 2008, but see Brown and Mitchell, 2001). Harder *et al.* (1992)

found that as a result of unilateral incompatibility between self-compatible *Erythronium albidum* and self-incompatible *E. americanum*, the seed set of both species was differentially affected by heterospecific pollen deposited. The presence of *E. americana* pollen on *E. albidum* stigmas significantly decreased its seed set, either through interference between pollen tubes or preemption of *E. albidum* by *E. americanum* pollen tubes. In contrast, mixed pollination did not decrease seed set in *E. americana*, probably because pollen tubes growing in conspecific styles were little affected by pollen of *E. albidum* on stigmas, and therefore most ovules were freely available for fertilization by conspecific pollen (Harder *et al.*, 1992).

Overall, despite many studies have found no detectable effect of heterospecific pollen on seed set, experimental evidence suggests that in some species and under certain circumstances, heterospecific pollen might strongly reduce seed set by stigma clogging, stigma closure, allelopathic inhibition and also by hybridization. As we showed in this section, hand pollination experiments are powerful tools to understand the underlying mechanisms of competition via HPD between co-flowering plants. Nevertheless, they may overestimate the importance of HPD in nature. For instance, amounts of heterospecific pollen grains received by flowers in hand pollinations (Galen and Gregory, 1989) were one-two orders of magnitude greater than the amount received in nature (Galen and Newport, 1988). Therefore, it is unlikely that the actual impact of HPD on these species is as high as that reported from hand pollination experiments (Galen and Gregory, 1989).

Furthermore, the few comparisons of amounts of con- and heterospecific pollen deposited in hand pollinations vs. natural populations, have shown that although the range of HPD in hand pollinations was much greater than in nature, conspecific pollen deposition in hand pollinations fall within the range found in nature (cfr. Caruso and Alfaro, 2000 vs. Caruso, 2000; and Waser and Fugate, 1986 vs. Kohn and Waser, 1985). Thus, results of hand pollination experiments should be taken with caution, because they might overestimate not only the absolute amounts of HPD, but also the relative magnitude and impact of heterospecific over conspecific pollen deposition occurring in natural conditions.

IV. ECOLOGICAL AND EVOLUTIONARY IMPLICATIONS

More than a century ago, Robertson (1895) suggested that flowering plant communities may be organized by negative interactions between plants utilizing the same pollinators, so that, with time, competition for pollination is reduced. This structure of species assemblages might be the outcome of two processes (or their combination), "ecological sorting"; that is the exclusion of less competitive species (Armbruster *et al.*, 1994), and the natural selection within interacting populations of phenotypic features that ameliorate competitive effects of neighbors, typically due to "character displacement" (Feinsinger, 1987). Models of

competition for pollination involving IPT predict that the less common of two competitors will go extinct if they are not separated along some dimension, or niche axis, that effectively reduces the level of pollinator sharing or IPT (Levin and Anderson, 1970; Waser, 1978a). All else being equal, we might expect that natural selection leads to divergence between species in characteristics related either to pollinator sharing (including habitat affinity, flowering time (on a seasonal or daily basis), morphology of floral parts related to pollinator choice), or to IPT by shared pollinators (like floral architecture, or pollen placement on pollinators' body), which represent different ways of a character displacement. Alternatively, competition avoidance might be achieved not by those types of divergence but by traits that confer independence of animal pollinators, like self-pollination (Rathcke, 1983). Above, we provided evidence on the existence of IPT in nature, and of its impact on plant's fitness. Nevertheless, before causal relationships between an ecological process (IPT) and interspecific patterns (character displacement) are invoked, the existence of a pattern should be conclusively demonstrated (Simberloff, 1983). Next, we review the evidence on species-pairs or community patterns consistent with the hypothesis of character displacement for reducing IPT.

A. Habitat Differentiation

Given that IPT is highly localized and sensitive to the density and dispersion of plant species (Rathcke, 1983), co-flowering sympatric plants sharing pollinators may avoid potential negative fitness consequences of IPT by habitat differentiation. The distance necessary to prevent pollen flow partly depends on flower relative density, pollen carryover—which is a measure of a donor's potential for siring seeds—and pollinators' flying capacity. If IPT leads to reduced seed set, species that are relatively rare may be most vulnerable to competition, because visits to rare flowers are likely to be followed by pollen-wasting visits to other species (Palmer *et al.*, 2003). Waser (1978a) estimated that the distance to avoid IPT might be in the order of meters or tens of meters, given the short distances over which the majority of pollen grains are dispersed in many systems. The outcome of simulations of IPT showed that spatial partitioning allows the coexistence of co-flowering sympatric plants that otherwise would experience competitive displacement through IPT (Waser, 1978a). A pattern consistent with the results of these simulations was found in *Asclepias verticillata* and *A. incarnatae* by Kephart (1983). These two plants, which experience substantial pollinator niche overlap and interspecific pollinator switches (Table 1), and experience reduced reproduction in synthetic mixed populations, grow in different habitat types in regions of sympatric distribution. Thus, the occupation of different habitats might allow co-flowering species sharing pollinators to avoid potential negative fitness consequences due to IPT.

B. Divergence in Flowering Phenologies

Competitive mechanisms involving IPT can be potential evolutionary forces that promote or maintain divergent flowering times (Waser, 1978b) through either the evolutionary displacement of blooming times or through an ecological sorting process that eliminates inferior competitors from communities leading to the coexistence of plant species that minimize flowering overlap. Consistent with this hypothesis of competition for pollination as a driver of phenological divergence, several studies of co-flowering species pairs sharing pollinators have provided evidence of decreased pollination during partial flowering overlap. For instance, through a set of field experiments, Campbell (1985b) found that the removal of nearby flowers of *Claytonia virginica* increased seed set in *S. pubera* (which blooms slightly later) only during the time of partial flowering overlap. In addition, *S. pubera* plants forced to bloom earlier were also more pollen-limited than control plants. In a subsequent paper, Campbell and Motten (1985) demonstrated that the primary mechanism of competition for pollination between those two species was CPL (see above), which constituted thus one of the selective forces acting on blooming time in *S. pubera* (Campbell, 1985b).

Within a stable assemblage of plants, consistent competition for pollination would be expected to result in nonrandom patterns among the species' flowering phenologies or morphologies, provided that no other constraints on those traits exist (Murray *et al.*, 1987). At the level of entire pollination guilds, and by comparing observed flowering phenologies with those obtained through randomization procedures, null model approaches have evaluated whether flowering phenology distributions are consistent with patterns of phenological displacement generated by pollinator-mediated competition.

After considering seasonality in flowering times in different null models, Aizen and Vázquez (2006) found overdispersed flowering times among plants pollinated by the hummingbird *Sephanoides sephaniodes* in the temperate forests of southern South America, a pattern consistent with the pollinator-mediated competition hypothesis. Evidence of depressed reproductive levels of most of those species during peaks of flowering overlaps, and confirmation that IPT occurs among some of them (Aizen and Vázquez, 2006 and references therein), suggest that IPT might be a potential mechanism partially generating such patterns.

Conversely, in a tropical cloud forest of Costa Rica, Murray *et al.* (1987) found no evidence of phenological displacement in either of two guilds of 19 and 17 plants pollinated by long-billed and short-billed hummingbirds, respectively. Although competition mechanisms certainly exist among some of the analyzed species pairs, the expression of such mechanisms in the field may actually be quite sporadic. Since neither consistent competition nor long stability characterizes many plant assemblages, these authors suggest that it is the temporally variable competitive environments which allow the coexistence of species (or

phenotypes), which have traits that would be suboptimal in stable environments (Murray *et al.*, 1987 and references therein).

Alternatively to the seasonal phenological divergence, partitioning of flowering times may be achieved on a daily basis. Many flowering species show substantial daily variation in stigmatic receptivity, pollen release and nectar secretion which might be interpreted as a way of avoiding IPT with other species sharing pollinators. Sympatric co-flowering *Acacia* species in Tanzania, for instance, show high intraspecific synchrony and interspecific segregated patterns of pollen release along the day (Stone *et al.*, 1998). The activity of shared pollinators is structured throughout the day accordingly with the temporal pattern of pollen release across *Acacia* species. The observed structuring of pollen release and pollinators activity has been attributed to pollinator-mediated competition (Stone *et al.*, 1998).

In spite of evidence of phenological displacement on a seasonal (Aizen and Vázquez, 2006) or daily (Stone *et al.*, 1998) basis, competition for pollination is not the only factor influencing phenological flowering patterns (Murray *et al.*, 1987; Aizen and Vázquez, 2006 and references therein). Thus, the presence of other forces tending to promote flowering synchrony and counteracting those that tend to reduce competition via shared pollinator, might explain partial flowering overlap in species suffering reduced fitness as a consequence of ITP (Waser, 1978b; Feinsinger 1987; Murray *et al.*, 1987).

C. Evolution of Floral Morphology

The hypothesis of divergence of floral morphology (“Sexual Architecture Hypothesis” *sensu* Murcia and Feinsinger, 1996) predicts that the severity of pollen transfer among flowering plant species should reflect their similarity in the placement of stigmas and anthers; that is, plants with divergent sexual architectures are expected to experience less IPT than plants with similar sexual architectures. Thus, IPT might be likely to promote morphological floral divergence among sympatric species (Kohn and Waser, 1985) through selection via female fitness on traits of focal plants that mechanically maximize conspecific pollen deposition (Caruso, 1999); such deposition is tightly related to the placement of pollen on the body of flower visitors.

The hypothesis of divergence of floral morphology among co-flowering sympatric species sharing pollinators as a way of avoiding IPT has received substantial support (Armbruster *et al.*, 1994; Caruso, 2000; Yang *et al.*, 2007; but see Schemske, 1981; Murcia and Feinsinger, 1996). Caruso (2000) found that selection on corolla length in natural populations of *Ipomopsis aggregata* via female fitness was significantly stronger in sympatric populations with the co-flowering species *Castilleja linearifolia*. Mechanical isolation was documented in two species of louseworts, *Pedicularis rhinanthoides* and *P. longiflora*, where stigmas pick up pollen from different parts of bumblebees, their exclusive pollinators (Yang *et al.*, 2007). As more indirect evidence, the lack of IPT in tropical nectarless *Cassia reticulatae* and *Cochlospermum vitifolium*, whose stigmas contact the ven-

tral and lateral sides of the bees’ abdomen, suggests that pollen from nectar-producing flowers visited by their pollinators is deposited elsewhere on the bee’s body (Snow and Roubik, 1987). Conversely, in spite of extensive IPT in the same location of pollinator bodies between sympatric co-flowering euglossine-pollinated *Costus allenii* and *C. laevis* (Zingiberaceae), these tropical herbs do not appear to experience competition for pollination (Schemske, 1981). Thus, divergence in floral morphology may prevent IPT in most situations; however, floral similarity and pollinator sharing might lead to facilitation of pollination in circumstances of extremely low plant densities, in which both species might function as Müllerian mimics with respect to food rewards (Schemske, 1981).

The divergence of floral morphology has also been tested at the community level. In a detailed field study, Armbruster *et al.* (1994) examined intra- and interspecific variation in three floral characters associated with the site of pollen placement on pollinators on 31 species of “triggerplant” (*Stylidium*) at 25 sites in Western Australia, in order to estimate “niche overlap” between sympatric species which flower nearly synchronously. Compared with null models of random assemblage structure, the frequency of niche overlap within each site was significantly lower than expected by chance. This study provided a clear demonstration of assemblage structuring and character displacement of floral traits as a way to allow several sympatric species to share pollinators without incurring either on significant pollen loss or receipt of heterospecific pollen (Armbruster *et al.*, 1994).

Conversely, in six plant species that vary in flower architecture but share the same hummingbird pollinator in the Costa Rican cloud forest, severity of CPL on floral structures other than anthers and stigmas was found to be independent of floral architecture (Murcia and Feinsinger, 1996). Short-tubed flowers adapted for short-billed hummingbirds often show similar pollen placement, which might suggest that they are more susceptible to IPT than long-tubed flowers. Thus, evidence on evolution of divergent floral morphology as a result of selective pressure exerted by IPT is equivocal and seems to be highly species or context-specific.

D. Spontaneous Selfing

Selection may favor species to forgo pollinator-dependent outcrossing to diminish the detrimental effects of heterospecific pollen (Geber and Moeller, 2006), or the wastage of pollen on heterospecific flowers. For example, the evolution of self-pollination in populations of *Arenaria uniflora* that co-occur with *A. glabra*, probably reflects selection against interference from heterospecific pollen, because pollination with *A. glabra* pollen results in nonviable seeds and reduces overall seed set (Fishman and Wyatt, 1999; Fishman, 2000).

Overall, we have got evidence for and against the general hypothesis of character displacement as a response to reproductive interference via IPT. One reason why clear-cut patterns of character displacement might be scarce in nature is that plants seldom coexist under stable conditions sufficiently for the competitive

demise of particular phenotypes or species (Feinsinger, 1987). Furthermore, the direction of selection itself may be quite inconsistent from generation to generation or from year to year, thus permitting the coexistence of species or phenotypes that would be maladaptive in a stable environment (Feinsinger, 1987). In addition, some mechanisms for competition among neighboring plants may be less important than had been previously supposed. In particular, HPD might occur in quite large numbers under certain circumstances (Table 2), but these heterospecific pollen grains may not always inhibit female reproduction in the ways proposed above (Table 3; Feinsinger, 1987).

V. IMPLICATIONS FOR CONSERVATION

A. Impact of Alien Flowering Plants on Natives' Pollination and Reproduction

In an extensive review on the disruption of plant reproductive mutualisms due to biological invasions, Traveset and Richardson (2006) identified the invasion of alien animal-pollinated plants as one of the major disruptors of plant-pollinator interactions. Furthermore, alien heterospecific pollen deposition on stigmas of native flowers, and, in the case of congenics, subsequent hybridization processes, were pointed out as two of the five possible mechanisms by which alien plants might disrupt pollination of natives (Traveset and Richardson, 2006; see also Bjerknes *et al.*, 2007).

As most invasive plants are generalists, they can easily integrate into plant-pollinator webs through interactions with local pollinators (Memmot and Waser, 2002; Aizen *et al.*, 2008), sharing pollinators with many native species (Morales and Aizen, 2002, 2006). In fact, field studies of mixed alien and native neighborhoods (Table 1) show high levels of both interspecific pollinator sharing (Grabas and Laverty, 1999; Moragues and Traveset, 2005; Larson *et al.*, 2006) and interspecific pollinator switches between invasive alien and native sympatric co-flowering species (Brown *et al.*, 2002; Totland *et al.*, 2006; Jakobsson *et al.*, 2008), suggesting a favorable scenario for IPT. Furthermore, as alien new species did not coevolve with the native plant community, much more IPT is expected because of the lack of evolution of mechanisms tending to reduce it (Jakobsson *et al.*, 2008).

When invasive plants reach high floral densities, alien pollen may virtually "inundate" the pollen transport webs at the community level, as demonstrated by the pollen loads carried by pollinators in flowering plants communities invaded by the Himalayan Balsam, *Impatiens glandulifera* in areas of natural and semi-natural vegetation in UK (Lopezaraiza-Mikel *et al.*, 2007). Since hand pollination experiments (Table 3) demonstrated an overall detrimental effect of alien pollen on seed set of some natives (Brown and Mitchell, 2001; Moragues and Traveset, 2005; Larson *et al.*, 2006; but see Jakobsson *et al.*, 2008), we might expect that this dominance of pollen transport webs by alien pollen translates in magnitudes of HPD enough to impair pollination. This remains to be tested.

Nevertheless, in most invaded areas, the amount of alien pollen naturally deposited on native stigmas seems to be rather low, suggesting that differences in pollen placement might avoid that high amounts of heterospecific pollen present in pollen vectors (e.g., Lopezaraiza-Mikel *et al.*, 2007) saturate the native stigmas. For instance, Jakobsson *et al.* (2008) found very low amounts of *Carpobrotus* invasive pollen in stigmas of native species growing in close vicinity (<1m), concluding that there is a low risk that transfer of *Carpobrotus* pollen affects their reproduction. Consistent with this, only one out of four studies that evaluated the natural deposition of alien pollen on native stigmas in mixed neighborhoods (Table 2) found a significant increase in alien pollen deposition (Ghazoul, 2002), while the other studies showed equivocal trends within and among studies (Grabas and Laverty, 1999; Moragues and Traveset, 2005; Larson *et al.*, 2006). Overall, amounts of alien pollen loads deposited on native stigmas by shared pollinators are not high enough to reduce reproduction by a mechanism of stigma clogging or saturation of stigmatic surfaces. Nevertheless, in cases of extreme relative abundance of alien flowers, detrimental effects on reproduction of natives seems inevitable as high amounts of alien pollen negatively affect the performance of native pollen (Table 3).

On the other hand, these studies showed that the presence of alien plants significantly reduced the amount of conspecific pollen deposition in some of the species pairs studied (Ghazoul, 2002; Larson *et al.*, 2006), while it had no detectable impact on others (Grabas and Laverty, 1999; Larson *et al.*, 2006) (Table 2). Thus, CPL might be an alternative mechanism by which alien flowering plants negatively affect pollination of natives. Furthermore, this mechanism might become central when alien flowers reach high densities and the number of alien flowers visited uninterruptedly increases.

A second, and less explored mechanism by which IPT with aliens may affect the pollination of natives is hybridization, which operates even with low levels of pollen transfer. Hybridization can lead to the extirpation of species, which may result when interspecific mating directly reduces the number of conspecific offspring and facilitates the incorporation of one parent's genome into that of another (Burgess *et al.*, 2008, and references therein).

Results of simulation models suggest that hybridization is perhaps the most rapidly acting genetic threat to endangered species (Wolf *et al.*, 2001). Smooth cordgrass (*Spartina alterniflora*) is a clonal perennial grass invader of salt marshes of the San Francisco Bay (USA), where it hybridizes with the common native California cordgrass (*S. foliosa*). Using values that reflect the underlying biology of the species to simulate the time to extinction of the native species as a result of hybridization, Wolf *et al.* (2001) found that *S. foliosa* would go extinct within 3–30 generations. In addition, they found that *S. alterniflora* or hybrids can invade a population even when this species is introduced in fairly low numbers, a risk amplified by rapid clonal growth, longevity and greater ecological amplitude of the invader and the hybrids (Wolf *et al.*, 2001).

The hybridization between the wind-pollinated native and endangered understory mulberry *Morus rubra* and the introduced invasive white mulberry *M. alba* in Canada (Burgess *et al.*, 2008) exemplifies the predictions of the simulation above into the real world. In sites where both species co-occur, 25% and 69% of total trees per plot are white mulberry and hybrids respectively, with only 6% of trees belonging to the native species. As a consequence, the overall availability of alien plus hybrid pollen vs. native pollen was 92% vs. 8%. This translated into 77% of *M. rubra* seeds being sired by hybrid pollen, 0% sired by alien pollen, while only 23% was sired by conspecific pollen. The percent of hybrids was significantly reduced when alien and hybrid plants were removed, which highlights the importance of the relative abundance of individuals, and thus of pollen availability on hybridization. Furthermore, as seed set of native species was unaffected by hybridization, the authors concluded that siring seeds by hybrid pollen occurs at the expense of those sired by conspecific pollen, a way of interspecific seed discounting (Burgess *et al.*, 2008). Given the high level of interspecific siring (77%), interspecific seed discounting by the introduction of an alien congener imposes a significant reproductive cost to the native *M. rubra*, which may potentially lead to its extirpation, as frequencies of native parental genotypes progressively decline. Hence, species in small populations might be particularly vulnerable to the direct impact of hybridization, and thus, native congeners of invasive alien species that reach high floral densities or produce high amounts of pollen are particularly at risk.

B. Transgene Introgression from Genetically Modified Crops to Wild Relatives

Pollen-mediated gene flow and hybridization among domesticated plants and their wild relatives has been a common phenomenon along the history of agriculture. Worldwide, many cultivated plants hybridize spontaneously with wild or weedy relatives (Snow *et al.*, 2003 and references therein). Nowadays, the field release of genetically modified or “transgenic” crops has raised concern about the possible introgression of genes from transgenic crops to related species. These concerns are scientifically justified because genetic exchange through IPT and interspecific hybridization is possible between a transformed organism and a nondomesticated organism, transgenes are inherited in the same way as naturally occurring genes, and they have the potential to persist indefinitely in cultivated or free-living organisms (Snow *et al.*, 2005).

There is a growing number of reported crosses between transgenic crops and wild relatives. For example, transgenic oilseed rape *Brassica napus* with herbicide resistance spontaneously hybridize with congeners like *B. rapa* (Jorgensen and Andersen, 1994) and *B. juncea* (Bing *et al.*, 1991), and under some circumstances it can also hybridize with species belonging to different genera within the Brassicaceae family, like *Sinapis arvensis*, *Raphanus raphanistrum* and *Hirschfeldia incana* (Chevre *et al.*,

1997; Darmency *et al.*, 1998; Darmency and Fleury, 2000). In spite of the wide range of potential hybrids from transgenic oilseed rape, the fecundity of the progeny varies among hybrids. For instance, introgression of *B. napus* genes into *H. incana* is not likely to be significant because of extremely low fertility of hybrids, whose seed output decreased in further generations (Darmency and Fleury, 2000). On the contrary, in spite of infrequent hybridization between transgenic *B. napus* and *R. raphanistrum*, successive back-crossed generations showed increased fertility, suggesting a quick increase of the number of resistant descendants (Darmency *et al.*, 1998). Thus, hybridization between transgenic crops and wild relatives has been demonstrated both in the laboratory and in the field, and there is evidence for the potential persistence of hybrid populations in the wild. The consequences of the introduction of these novel traits into wild populations are still beginning to be understood.

The most widespread transgenic crops are those genetically modified for herbicide resistance (e.g., glyphosate-tolerance) (Snow *et al.*, 2005 and references therein). Therefore, one of the major risks imposed by crop-to-wild transfer of resistance genes is the evolution of herbicide resistance in wild relatives or the increase of herbicide resistance of crop weedy relatives. This might have unforeseen economical and ecological consequences, like the need of applying more powerful herbicides to fight novel “super” resistant weeds.

Other transgenic trait widely introduced to field-released crops is resistance to insect pests (e.g., *Bacillus thuringiensis* [Bt] transgene), which, if transferred to wild plants might reduce insect damage, and in turn increase fitness. For example, Bt transgenes can be transferred from cultivated transgenic sunflower (*Helianthus annuus*) to wild weedy sunflower (*H. annuus*) via pollination, which can increase the seed set of Bt wild hybrids by 55% (Snow *et al.*, 2003). Given the fecundity advantage of transgenic hybrids compared to wild nontransgenic plants, selection can strongly favor an increase in the frequency of Bt transgene within the wild sunflower population (Snow *et al.*, 2003). Thus, the transfer of resistance against insect herbivory to wild plant species might disrupt plant-herbivore interactions, with potential cascading effects to biotic communities.

A third and probably underestimated risk of the field release of transgenic crops is the transfer of transgenes from genetically modified crops to wild relatives of domesticated crops and traditional landraces, in particular in centers of crop origin, diversification and domestication (Mercer and Wainwright, 2008 and references therein). The genetic diversity found in landraces of major crops in centers of origin comprises one of the world’s most important natural resources for future plant breeding (Altieri *et al.* 1987), and have an immeasurable cultural value for local people that have grown these landraces along generations. The introgression of transgenic traits might erode the genetic integrity and diversity of these landraces, which in turn might threaten both local food autonomy and global food security.

Currently, it is impossible to prevent gene flow between sexually compatible species that inhabit the same region because pollen disperses too easily to make complete reproductive confinement practical. Therefore, preventing the field release of genetically modified crops, in particular in regions where wild or weedy related species occur, or in centers of high diversity of local landraces seems the only way to avoid transgene spreading via interspecific pollen transfer, and to prevent unwanted environmental and socioeconomical consequences.

VI. CONCLUDING REMARKS

Interspecific pollinator sharing appears to be rather common among co-flowering sympatric plants (Table 1). Nevertheless, as switching between flowers of different species is a necessary condition for IPT to occur, it is not surprising that a minor fraction of the species within a flowering guild experience high and consistent levels of IPT with other guild mates.

Most studies give circumstantial evidence for the existence of HPD and CPL, and, therefore, we badly need more data that compare natural levels of conspecific and heterospecific pollen deposition on stigmas of focal plants growing in the presence (mixed neighborhood) vs. the absence (pure neighborhood) of a second co-flowering species. With the information gathered to date, IPT seems to be ubiquitous, but its overall magnitude does not seem to be consistently high, what has been attributed by some authors to the effect of high pollinator constancy. Nevertheless, other factors beyond floral neighborhood and pollinator constancy may affect the dynamics of pollen transfer within and among species.

Of the two components of IPT, heterospecific pollen deposition (HPD) on stigmas has received more attention than the other component, conspecific pollen loss (CPL), despite that HPD and CPL are two facets of the same process. Since HPD requires a relatively finer matching between reproductive parts of both co-flowering plants, it seems to be less ubiquitous than CPL. Furthermore, evidence from field studies suggests that both the magnitude and the impact of CPL on plant reproduction are more important than those of HPD. Such pollen loss has been shown to reduce maternal fitness by decreasing not only the number of offspring (Campbell 1985b; Campbell and Motten, 1985; Feinsinger *et al.*, 1991) but also its quality (Bell *et al.*, 2005).

The amount of pollen removed from a flower is usually very difficult to quantify (Feinsinger, 1991), but it has been assumed that removal is positively correlated with pollen deposition (Snow and Roubik, 1987 and references therein). Therefore, to date, CPL has been indirectly estimated from levels of conspecific pollen deposition and its impact has been measured in terms of seed set, which reflects female reproductive success. Nevertheless, CPL might actually affect male reproductive success more strongly than available estimations show (Feinsinger 1991). Future research on the impact of CPL on plants' reproduction should thus make a special effort to obtain accurate measures of CPL that reflect the plant's male perspective.

Studies involving hand pollination experiments have been useful to evaluate the effect of heterospecific pollen on plant reproduction, as well as its underlying mechanisms, although results from those experiments should be taken with caution because they might overestimate both the magnitude of HPD and the relative importance of heterospecific over conspecific pollen deposition in nature. Moreover, a high incidence of HPD in nature may not necessarily impair reproduction if heterospecific pollen does not alter the performance of the own pollen.

From such studies, alternative mechanisms proposed to explain how HPD can depress reproduction acting at different stages of the pollination and fertilization process (i.e., physical saturation of stigmas, mechanical clogging, allelopathic inhibition of conspecific pollen germination, inhibition of pollen tube's growth, or usurpation of ovules) have found experimental support. Laboratory experiments showed that in some combinations of pollen donor and recipient species, amounts of heterospecific pollen as small as those occasionally found in the field might strongly reduce maternal fitness through allelopathic inhibition, usurpation of ovules or hybridization. Furthermore, these mechanisms haven shown to have substantial impact on plant reproduction, and on plant-to-plant interactions via pollination in the field (Jorgensen and Andersen, 1994; Murphy and Aarsen, 1995; Burgess *et al.*, 2008). However, with the available information, we cannot yet assess the specific circumstances under which one mechanism will prevail over the others.

Regarding the interpretation of the results of hand pollinations, some counterintuitive positive effects of heterospecific pollen grains on seed set might arise from a positive relationship between the amount of conspecific and heterospecific pollen, if for instance, stigmatic receptivity affects pollen adherence. Therefore, future hand pollination studies should take into account not only seed set, but also the total amount of conspecific and heterospecific pollen deposited on stigmas in each treatment.

For more than a century, a broad range of studies have examined how competition for pollinators among flowering plants could be a major force promoting evolutionary divergence in characteristics related to pollination, such as habitat affinity, flowering phenology, and floral architecture (morphology of floral parts related to pollinator choice, or differential placement of pollen on the bodies of visitors). Null models have proved to be a powerful tool to address this kind of research question (Aizen and Vázquez, 2006) and future research including phylogeny in this methodological approach is highly promising. Different studies have found strong or partial evidence for the importance of competition mediated by interspecific pollen transfer on each of those characters, while others have failed to do so. Overall, divergent patterns of flowering phenologies and floral morphologies as a consequence of conspecific pollen loss-mediated competition have received the strongest support.

Finally, the evolutionary, environmental and economical consequences of interspecific pollen transfer are currently receiving special attention, as the introduction of alien plant species or the

release of transgenic crops are becoming increasingly common. Overall, studies on these fields suggest that despite HPD having a minor and probably negligible role in pollinator-mediated competition among plant species, it might have profound ecological and evolutionary impacts on biotic communities.

The recent work dedicated to evaluate the impact of alien pollen deposition on native stigmas by shared pollinators show that under natural conditions, such impact is overall rather low. In cases of extreme relative abundance of alien flowers, however, detrimental effects on reproduction of natives seems inevitable. In particular, pronounced negative effect of alien pollen via hybridization may be found in native species closely related to alien invaders. This mechanism of hybridization has been found to operate even with low levels of pollen transfer, and may represent a serious threat to endangered species, leading even to their extirpation from native communities (Wolf *et al.*, 2001; Burgess *et al.*, 2008). Therefore, species in small populations are those apparently more vulnerable to such effect. The most effective, economical and ecologically sound approach to managing invasive species is to prevent them from arriving (Windle and Chavarría, 2005). In addition, monitoring and control of established invasive flowering plants is crucial to prevent them from spreading into areas where native related species occur.

More concerning, there is growing evidence supporting the claim that the field releases of transgenic crops might lead to transgene flow to wild relatives via ITP. The spread of novel transgenic traits in nature can disrupt ecological interactions and alter the functioning of the native communities and agroecosystems, with unforeseen and undesirable ecological, evolutionary and socioeconomic consequences. Therefore, in the face of the growing development and release of novel genetically modified organisms, more interdisciplinary research, risk assessment, monitoring and a strong regulatory framework is urgently needed, together with precautionary actions regarding further field release of transgenic crops.

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