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Genetic and Ecological Consequences of Transgene Flow to the Wild Flora

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Abstract Gene flow from crops to wild relatives by sexual reproduction is one of the major issues in risk assessment for the cultivation of genetically engineered (GE) plants. The main factors which influence hybridization and introgression, the two processes of gene flow, as well as the accompanying containment measures of the transgene, are reviewed.

The comparison of risks between Switzerland and Europe highlights the importance of regional studies. Differences were assessed for barley, beet and wheat. Moreover, transgene flow through several wild species acting as bridge (bridge species) has been up to now poorly investigated. Indeed, transgene flow may go beyond the closest wild relative, as in nature several wild species complexes hybridize. Its importance is assessed by several examples in Poaceae. Finally, the transgene itself has genetic and ecological consequences that are reviewed. Transgenic hybrids between crops and wild relatives may have lower fitness than the wild relatives, but in several cases, no cost was detected. On the other hand, the transgene provides advantages to the hybrids, in the case of selective value as a Bt transgene in the presence of herbivores. Genetic and ecological consequences of a transgene in a wild species are complex and depend on the type of transgene, its insertion site, the density of plants and ecological factors. More studies are needed for understanding the short and long term consequences of escape of a transgene in the wild.

Keywords Risk assessment · Transgene · Genetically engineered plants · Bridge species · Switzerland

Abbreviations

GE	genetically engineered
FOEN	Swiss Federal Office for the Environment

1
Introduction

The gene transfer from crops into populations of wild relatives has become an important scientific and public issue since the development and cultivation of genetically engineered (GE) plants in the late 1980s [1]. The concerns related to the cultivation of GE crops, in particular those dealing with the possibility of transgene escape into the wild flora, have generated a multitude of studies on crop-to-wild gene flow [1–9]. While these studies have shown that such gene flow exists for almost all of the most important crops cultivated worldwide, only recently have new studies focused and are focusing on its ecological and genetic consequences [9–11]. Yet, in order to better assess the ecological and agronomic risks associated with the transgene flow to the wild flora, it is fundamental to understand the mechanisms and the consequences of such gene flow [12].

Studies on the existence of crop-to-wild gene flow have already been reviewed several times in the context of the cultivation of GE crops (e.g. [2, 5, 7]). A general overview on the factors influencing gene flow, and containment measures is presented here. Risk of gene flow has a geographical component and we focus in Sect. 2 on the particular case of Switzerland. While gene flow has been mostly investigated from crops to their closest wild relatives, further introgression may occur between wild species. The importance of such "bridge species" is explained in Sect. 3. Finally, the genetic and ecological consequence of transgene flow is evaluated in Sect. 4.

1.1
Factors Influencing Gene Flow

It is widely accepted that hybridization between two taxa depends on several key factors, such as their sympatry, the synchrony of their flowering periods, the existence of a common vector for the gametes, as well as their reproductive compatibility and the viability and fertility of the hybrids [12]. Generally speaking, gene flow between two taxa can thus be viewed as a two step process: (i) a first hybridization event, which leads to the production of first generation hybrids, followed by (ii) the introgression of part of the genome of one species into the other by successive backcrosses.

Hybridization depends mainly on straightforward conditions, such as the need for the plants to grow close to each other and the potential for the exchange of pollen. While the most obvious situation where both conditions are met is represented by cultivated fields where the wild relatives grow in close proximity, it is worth noting that crop plants growing as volunteers within fields of other crops or in other habitats represent additional contact zones between crops and wild relatives. In the case of GE crop, such a situation may lead to transgene escape to the wild flora. A notable example of this latter situation is that of rapeseed, which is extremely common to see in any kind of disturbed habitats even relatively distant from cultivated areas [6].

More generally, the establishment of feral crop populations in the agroecosystems, as well as outside the cultivated areas depends mainly on the crop features, such as seed dispersal by wind, water or animals, absence of dormancy, ripening period, persistence of seeds in the soil [13, 14]. Agricultural practices (harvesting period, crop rotation, till vs. no-till) as well as post-harvesting procedures (transportation), can also greatly influence the emergence of volunteer plants.

Finally, an additional potential source of transgenes is represented by first and subsequent generations of hybrids between GE crops and wild relatives, which can act as "genetic bridges" between the parental species [5, 9].

1.2
Factors Influencing Hybridization

Hybridization is influenced quantitatively by numerous factors, some of them depending on the characteristics of the plants, while others are more related to the environment. Hybridization is frequent in perennial species and especially for outcrossing and clonal plants [2], as the produced hybrids can subsist clonally even in the case of reduced fertility.

Pollen vectors play a major role, at least on the distance at which hybridization can take place. For instance, maize pollen is known to be particularly heavy and intraspecific gene flow at distances greater than 50 meters is unlikely [15]. In contrast, other wind-pollinated species can show large distance pollen dispersal events. Watrud et al. [16] discovered intraspecific hybrids of *Agrostis stolonifera* 21 km from the pollen source.

While it is obvious that topography influences winds, a flat land favoring the pollen flow over long distances, it is worth mentioning that microtopography seems to have also an impact on the behavior of pollinator insects, by hiding or making more visible potential pollen sources and sinks. However, predictions on the pollen movements seem more complicated in the case of insect pollinated species. Different experimental and modeling studies on the distance at which rapeseed pollen could produce hybrids, generated indeed inconsistent results [17, 18], because these results depend indirectly on the factors influencing the activity of bees [19].

Repeated contacts with crop populations are known to accelerate the introgression process [20]. However, hybridization as well is positively correlated with the frequency and the extent of the contact zones between crops and their wild relatives. Indeed, feral populations or individual volunteer crop plants will not only increase the area of contact, but also increase the potential for the overlap of flowering periods. For instance, while in central Europe fields of rapeseed usually flower simultaneously in May, it is common to observe volunteers flowering from June till late October.

1.3
Factors Influencing Introgression

Most factors influence both hybridization and introgression. While successful introgression is achieved when genes from one taxon are fixed in another one, several hybrid generations and parental individuals can be involved in the process. All of these individuals and generations can coexist and exchange genes simultaneously for many years [5].

Fitness of hybrids is essential to successful introgression. Moreover, independent of the pollen vector, the intensity and symmetry of pollen flow will determine both the direction of hybridization and the speed of introgression in a sink population. Fixation of genes is known to occur more rapidly in small populations, which are also more prone to act as a pollen sink [21, 22].

Both hybridization and introgression are facilitated in genetically close species, such as crop and prickly lettuce (D'Andrea et al., unpublished) or crop and wild sunflower [23], rather than between more distantly related species like rapeseed and wild radish [24]. The actual introgression of crop genes into the genome will depend greatly on the existence of pre- and postzygotic barriers, which strongly depend on factors linked to the evolutionary divergence between the crop and its wild relative, the incompatibilities being generally higher between genetically distant taxa and lower between closely related taxa.

Genetic barriers acting against hybridization between species are considered by several authors as "semi-permeable" [25, 26]. Individual genes or specific genome regions may be transferred during introgression processes, rather than entire genomes [5]. Moreover, genes from one species may not be uniformly transmitted to another via introgressed generations, as selection does not act homogeneously within genomes.

A factor that influences specifically introgression, rather than hybridization, is the observation that beneficial or neutral traits will be preferentially introgressed, compared to detrimental genes. For example, silenced genes can be kept in recipient genomes, until they are eliminated by genetic drift [23]. Additionally, several linked genes may be transferred together, especially if such complexes carry positively selected genes.

The situation is more complex in polyploids where multiple copies of genes make genetic interactions even more complicated. Moreover, related polyploid species often share only part of the genome (e.g *Triticum aestivum* and *Aegilops cylindrica*, *Brassica napus* and *B. campestris*) and introgression from one species to the other is easier for genes located on the homologous chromosomes, than for genes located in the homeologous ones.

1.4
Containment of Transgenes

One clue which arises from the existing studies on crop-to-wild gene flow is that hybridization between most crops and their wild relatives cannot be avoided [4]. Therefore, if the goal is to impede the transfer of transgenes to the wild flora, gene flow has to be stopped at its source. For this purpose, several strategies, each possessing advantages and drawbacks, have been proposed (most are reviewed in [5]), which are mostly linked to the mechanisms and factors influencing introgression presented above.

Since physical barriers, such as isolation by distance or hedge rows bordering fields appeared rapidly to be inefficient, genetic barriers based on the breeding systems of the crops were investigated. One of the first ideas was to decrease or completely block gene flow via pollen, by favoring apomixis. However, many apomictic species preserve low to moderate sexual seed production, and moderate or high levels of pollen [14].

It was thus suggested to induce male-sterility in GE crops. This system was applied to commercialized *Brassica napus* varieties [27]. In this rapeseed variety, the transgenic construct is induced by a *tapetum*-specific promoter, and produces a cytotoxin (*barnase*). Only anthers express the lethal transgene, which leads to the destruction of the mother cells of pollen. However, male sterility does not prevent the formation of hybrids when wild relatives act as paternal parent, like in the case of bolting beets in south Europe [4]. Moreover, these two strategies can only prevent gene flow by pollen, while they have no effect on gene flow by seeds.

It was subsequently suggested to insert transgenes in genomic regions, which have no or reduced mobility. As mentioned previously, genomes are not uniformly transmitted, and some regions are more “mobile” than others [25, 28]. Targeting gene insertion in regions poorly transmitted should decrease the probability of gene escape. However, in order to be efficient, this strategy has to be developed on a case-by-case basis, and introgressive patterns on all possible wild relatives of each crop should be known. A similar idea was proposed for polyploid species, where genomes non-shared by wild relatives could be chosen as insertion sites of transgenes (e.g. [29, 30]). However, recombination events between non-homeologous genomes were observed in wild x crop hybrids involving *Brassica napus* [31], and *Triticum aestivum* [32, 33].

Another proposition was to insert transgenes in the DNA of mitochondria or chloroplast, as organellar DNA is usually maternally transmitted, and should not be carried by pollen grains in Angiosperms [34]. However, paternal inheritance of chloroplasts has already been observed (reviewed by [35]). For instance, transfer of genes from organelles to nucleus occurs at a low frequency in tobacco, as one pollen grain out of 16 000 carries cytoplasmic genome elements in its nucleus [36]. As for the strategies presented above, gene flow via seeds is not prevented.

Therefore, so-called “seed suicide” techniques were proposed (see [12] for a review). In these plants, the transgenic construct induces the production of lethal protein or blocks physiological functions during seed maturation, which makes it impossible for the seeds to germinate, but without disturbing albumen differentiation. However, producing non-germinating seeds would impede farmers from sowing part of their harvest, which is a highly controversial issue from an ethical point of view.

Another recent technique consists of the chemically induced removal of transgene from pollen cells during the gametogenesis. The transgene is flanked by specific sites (*lox*), which allows its removal by a site-specific recombinase (Cre). The recombinase is coded by the transgene and expressed after induction [37]. Recombinase-based techniques present currently two major drawbacks: the controlling system has to be activated by an external signal, that is the application of tetracycline, and basically every single cell involved in the sexual reproduction of the crop should be treated.

Finally, post-hybridization and fitness-based strategies were also suggested to avoid the spread of hybrid derivates in the environment. The idea is to lower the fitness of these plants by linking the transgene with traits which are neutral or beneficial in an agricultural context, but detrimental in the wild. The genes responsible for traits such as dwarfing, loss of dormancy or non-shattering of seeds were proposed as suitable loci to place transgenes [5]. However, there are at least two serious drawbacks in this strategy. First, the current technology does not allow placing of the transgenic construct in a precise location. Second and probably more important, most of these so-called deleterious traits are recessive loss-of-function alleles related to the domestication of crops [13]. These alleles would thus not be expressed in first generation hybrids with a wild plant, because of the presence of the dominant wild allele in their genome. In further generations, the deleterious allele would only be expressed in homozygous individuals, which would strongly reduce its capability to lower the fitness of these plants. Moreover, if the hybrids are fertile, this strategy would not prevent them acting as a genetic bridge and pollinating the wild parent [11].

Alternatively, this strategy could have a good efficacy when the transgene is coupled within the transgenic construct itself with one or two mutant genes conferring an ecological disadvantage (transgenetic mitigation, [38]), such as dwarfing, as demonstrated in tobacco introgressants [39].

2

Gene Flow between Cultivated Plants and Wild Relatives: the Case of Switzerland

Risks related to transgenic plants are often investigated on a worldwide scale and several reviews have focused on this topic. Nevertheless, a regional perspective is necessary because crops vary among countries, wild species have often a limited geographical range and floras composition changes geographically. Consequently, the distribution of crops and their ability to cross with their wild relatives vary regionally. Moreover, the genetic characteristics of a wild species, as for example its ploidy level, may vary according to their geographical range and can influence largely their ability to hybridize. This is illustrated for example by tetraploid alfalfa, *Medicago sativa* ($2n = 32$ chromosomes). In Switzerland, its wild relative, *Medicago falcata*, is tetraploid and has the same chromosome number ($2n = 32$) except in Unterengadin, where it is diploid ($2n = 16$). Hybrids between the two species, *M. x varia*, are found frequently where both species are tetraploid, but are, on the contrary, very rare in the range of the diploid *M. falcata* [40]. Risks of gene flow are consequently much lower in Unterengadin than in the other areas of Switzerland.

Consequently, the results of one country cannot be necessarily generalized to another country without further investigations. This is particularly true for Switzerland, where topography strongly influences the distribution of wild species and constrains agriculture. Its landscape typically illustrates that risks may vary from one area to the other.

Distribution of wild relatives may also vary in time. For example, global change, including both the global warming and the increase in disturbance as a consequence of human activity, has led to the northern expansion of several Mediterranean species. Similarly, change in agricultural practices may influence the contact zones between crops and their wild relatives, and consequently influence greatly the risks. Therefore, monitoring over a long term the wild flora and the agricultural areas is necessary in order to evaluate the risks on a regional perspective. Switzerland has voted on November 27, 2005 a moratorium of 5 years on the outdoor cultivation of GE organisms for commercial purposes. Probabilities of large-scale cultivation of transgenic plants are therefore low. Nevertheless, political changes may occur rapidly and therefore, assessment of potential risk of gene flow from crops to wild relative is necessary with a Swiss perspective.

The Swiss Federal Office for the Environment (FOEN) granted a study on risk assessment which focused on the main cultivated plants of Switzerland. For each of them, bibliographical data were collected on the crop and on most of the wild relatives (Table 1). From this, the risks of transfer of transgene to conventional varieties and to the wild or naturalized flora were evaluated (Table 2).

Risks of gene flow were never null between cultivars, as all crops reproduce sexually. Risks were evaluated as null for the wild flora when the crop produces no feral populations and no wild relative exists in Switzerland. It was low to medium in the case of autogamy, or of harvest before flowering (as for lettuce, out of the seed production areas). Risk was considered as high for all allogamous species, those forming spontaneous or subspontaneous populations, or possessing wild relatives that hybridize readily with the crop.

Risks may be examined in different perspectives. For a monitoring program, the priority is to examine commercialized transgenic crops. Prior to the authorization of outdoor cultivation, it is important to evaluate on one hand the risks of contamination of non-transgenic cultivation, and on the other hand those of gene flow to the wild flora.

Table 1 Characteristics collected for the crops and its wild relatives

Common for the crops and its wild relatives	Specific to the cultivated plant	Specific to the wild relatives
Latin name	Extent of cultivation	Ecology
Vernacular names	Feral populations	Hybridization with the crop
Chromosome number	Frequent transformations which have led to a request for a field trial	Hybridization with other wild relatives
Pollen dispersal	Recent transformation	Category of threat according to the Swiss Red List [104]
Breeding system	GE field cultivation	Stability of the distribution
Longevity	Commercialization	
Levels of vegetation		

Table 2 Summary of risks for the main crops cultivated in Switzerland

Name		Crop to crop gene flow*	Gene flow with spontaneous and naturalized flora*		Commer-cialization**	Main transformation
			Suisse	Europe		
Poaceae						
Agrostis stolonifera L.	Creeping bentgrass	++	++	++	pending	Herbicide tolerance, agronomical properties
Avena sativa L.	Oats	+	++	++	FT	Virus Resistance (virus BYDV
Cynodon dactylon (L.) Persoon	Bermuda grass	++	+	+	FT	Agronomical properties, herbicide tolerance
Festuca arundinacea Schreber s.l., *F. pratensis Hudson s.l.*	Fescue	++	++	++	FT	Product quality, fungal resistance
Hordeum vulgare L.	Barley	+	0	+	FT	Product quality, fungal resistance
Lolium perenne Hudson s.l., *L. multiflorum* Lamarck	Ryegrass	++	++	++	FT	Product quality
Poa pratensis L.	Smooth meadow-grass	++	++	++	FT	Herbicide tolerance
Triticum aestivum L., *Triticum spelta* L.	Wheat	+	+	++	FT	Herbicide tolerance, fungal resistance
Zea mays L.	Maize	++	0	0	Com	Insect resistance, herbicide tolerance

Table 2 (continued)

		Crop to crop gene flow*	Gene flow with spontaneous and naturalized flora*		Commer-cialization**	Main transformation
Name			Suisse	Europe		
Rosaceae						
Fragaria x ananassa	Strawberry	+	+	+	FT	Fungal resistance, herbicide tolerance
Malus domestica Borkh.	Apple	++	++	++	FT	Product quality (fruit quality), insect and bacterial resistance
Prunus avium L.	Cherry	++	++	++	FT	Modification of metabolism
Prunus domestica L.	European plum	++	++	++	pending	Virus resistance
Pyrus communis L.	Pear	++	++	++	FT	Product quality (fruit maturation)
Rubus idaeus L.	Raspberry	++	++	++	FR	Virus resistance, product quality
Asteraceae						
Cichorium intybus L.	Chicory	++	++	++	Com	Agronomical properties (male sterility), herbicide tolerance
Helianthus annuus L.	Sunflower	++	+	+	FT	Fungal resistance, insect resistance
Lactuca sativa L.	Lettuce	+	+	+	FT	Herbicide tolerance, product quality

Table 2 (continued)

Name		Crop to crop gene flow*	Gene flow with spontaneous and naturalized flora*		Commercialization**	Main transformation
			Suisse	Europe		
Fabaceae						
Glycine max L.	Soybean	+	0	0	Com	Herbicide tolerance, product quality
Medicago sativa L.	Alfalfa	++	++	++	Com	Herbicide tolerance, product quality
Pisum sativum L.	Pea	+	+	+	FT	Herbicide tolerance, virus resistance
Solanaceae						
Lycopersicon esculentum Miller	Tomato	+	0	0	Com	Product quality, insect resistance
Nicotiana tabacum L.	Tobacco	++	0	0	Com	Product quality, virus resistance
Solanum tuberosum L.	Potato	+	0	0	Com	Insect resistance (doryphore), product quality
Other families						
Beta vulgaris L.	Beet	+	0	++	Com	Herbicide tolerance, virus resistance
Brassica napus L.	Rapeseed	++	++	++	Com	Product quality (oil quality), herbicide tolerance
Brassica rapa L.	Rape	++	++	++	FT	Insect resistance (lepidopters), herbicide tolerance
Cucumis melo L.	Melon	++	0	0	FT	Virus resistance, product quality (fruit ripening)

Table 2 (continued)

Name		Crop to crop gene flow*	Gene flow with spontaneous and naturalized flora*		Commer-cialization**	Main transformation
			Suisse	Europe		
Cucumis sativus L.	Cucumber	++	0	0	FT	Virus resistance, agronomic properties (salt tolerance)
Cucurbita pepo L.	Squash	++	0	0	Com	Virus resistance
Daucus carota L.	Carrot	++	++	++	FT	Fungal resistance (*Alternaria* tolerance), product quality
Dianthus caryophyllus L.	Carnation	++	0	0	Com	Product quality (colors modification)
Osteospermum ecklonis (DC) Norl	Cape Daisy	++	0	0	FT	Metabolism modification
Picea abies (L.) Karsten	Norway Spruce	++	++	++	FT	Gene marker
Pinus sylvestris L.	Scots pine	++	++	++	FT	Gene marker, forestry performance
Populus alba x tremula, *Populus* sp.	Poplar	++	+	+	FT	Herbicide tolerance, forestry performance
Vitis vinifera L., *Vitis labrusca* L.	Grape	++	+	+	FT	Fungal resistance, virus resistance

* Evaluation of risks: 0 = no risk, + = low or medium risk, ++ = high risk

** Commercialization: FT = field tests have been carried out; pending = commercialization of transgenic varieties is pending; Com = transgenic varieties are commercialized

2.1
Priority Species for a Monitoring Program

Eleven cultivated species in Switzerland possess commercialized transgenic varieties elsewhere in the world. Six of them present a high risk of gene flow with other cultivars (alfalfa, carnation, chicory, maize, rapeseed and squash). Others represent a lower risk as they are harvested before flowering, such as beet, or because seed do not mature in the regions, such as potato for example. Moreover, crops with an autogamous breeding system such as soybean, tobacco or tomato also present a lower risk of gene flow.

2.2
Potential Risks for the Contamination of Non-Transgenic Crops

Only crops which have commercialized GE varieties are mentioned below. The higher risks originate from the six allogamous species mentioned above. Medium risks are characteristics from either autogamous species with partial allogamy, or those which are not producing fruits in traditional practices (beet, potato). Low risks exist for plants that do not flower, when vegetative parts are collected. Such cultivation necessitates a good management and strict control, in order to avoid any loss of seeds or unintended flowering. Some of the species mentioned above belong to that category, depending on their use.

2.3
Potential Risks for Gene Flow to the Wild or Naturalized Flora

High risk characterizes crops and wild relatives with no or low reproductive barriers, as for oilseed rape and creeping bentgrass. For example, escape of transgenic creeping bentgrass (*Agrostis stolonifera* L.) in non-agronomic areas was observed in the USA [41]. Medium risk occurs if the hybrid is partially fertile and introgression is possible. No commercialized transgenic crops belong to that category: pea, poplar, strawberry, sunflower and wheat. Some cultivated species have no wild relative in Switzerland; this is for example the case for beet, carnation, maize, melon, potato, soybean, tobacco, tomato and squashes. Consequently, they do not represent a genetic threat for the natural flora, even if containment measures are needed to avoid crop to crop gene flow.

2.4
Particularity of the Swiss Flora

Table 2 reveals that, for some species, different risks were assessed between Switzerland and Europe. Barley present no risk for Switzerland, as no ancestor grows in this country [42], while in the eastern Mediterranean to Iran and West Central Asia, hybridization occurs readily with *Hordeum spontaneum* [4]. Wheat presents also a lower risk in Switzerland, where only *Ae. cylindrica* forms durable populations, contrasting with the Mediterranean area where several wild relatives of *Aegilops* are frequent. Finally, beet presents no risk of outcrossing with the wild flora because its wild relative *Beta vulgaris* subsp. *maritima* is absent in Switzerland, while it is present close to the Atlantic coast and along the Mediterranean boarder.

3
The Importance of Bridge Species

Historically, the term "bridge species" has been used to designate wild plant species which could act, through artificial or natural hybridization, as a genetic bridge between wild relatives and closely related cultivated plants. Figure 1 shows that there are potentially three possible directions of the gene flow through bridge species: (1) wild-to-crop bridges, (2) crop-to-wild bridges and (3) wild-to-wild bridges.

The wild-to-crop bridges have been used by humans since millennia and are still used by breeders for the introduction of desirable traits from wild relatives into crops [43]. As discussed above, the development of GE crops has brought much more attention to the gene flow the other way around, that is between cultivars/crops and their wild relatives [2, 5, 7]. Surprisingly, the potential further spread of transgenes to other wild relatives via wild-to-wild

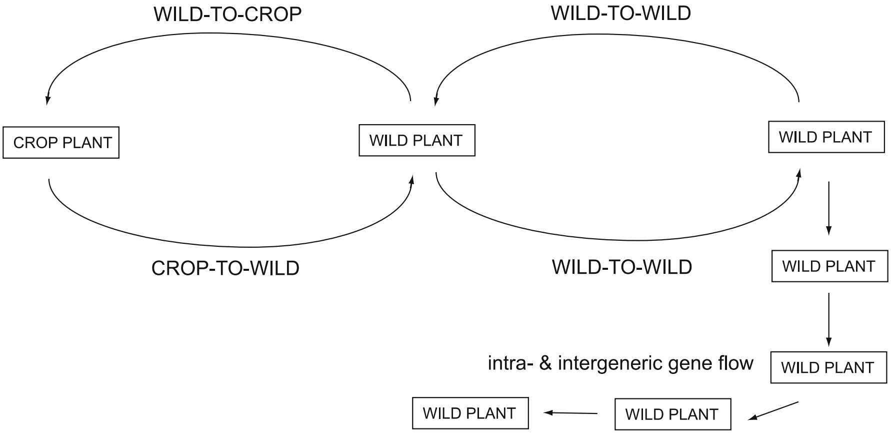

Fig. 1 Bridge species and directions of gene flow in crop-wild hybrid complexes. The potential spread of transgenes into wild populations via wild-to-wild bridges and further introgression has been poorly investigated

bridges and so-called stepping-stones introgression has been rarely if ever studied in details in the GE plants context. Thus, after a short description of the first well studied and described gene flow direction, more attention will be devoted to the still weakly explored subject of wild-to-wild bridges.

3.1
Wild-to-Crop Bridges

The term "bridge species" designates here wild relatives of cultivated plants which are used during artificial and/or natural hybridization procedures for crop improvement to circumvent some experimental or environmental constrains (Fig. 1). The ability to transfer genes between related plant species has been a great benefit in the improvement of cultivars for disease resistance, insect resistance, and/or end-use quality. This has been especially true in allopolyploid crops where there are multiple species that can act as donors. The best documented examples come from studies of gene transfer from wild species to wheat (*Triticum aestivum*). Romero et al. [44] obtained for example the transfer of a cereal cyst nematode resistance gene from *Aegilops triuncalis* (donor) to hexaploid wheat using bridge species *T. turgidum*. Fernandes et al. [43] transferred to wheat stem and leaf rust as well as powdery mildew resistance from *Ae. squarrosa* (donor) through hybridization with *T. durum* (bridge species). Such methods imitate in fact the ancient hybridization events, which happened during evolution and domestication of some crop plants, e.g. the hexaploid wheat. This bridge species method with development of intermediate natural or artificially synthesized amphiploid hybrids is one of the available procedures to facilitate gene flow between wild relatives and crop. It has been used for many decades not only for wheat cultivars [45] but also for many other crops (e.g. *Brassicaceae* [46], *Gossypium* sp. [47], *Cucumis* sp. [48]). However, for numerous plant groups such approaches are very laborious and/or have low or no success (e.g. for some *Solanum* sp. [49]).

3.2
"New Old Issue": Wild-to-Wild Bridges and Stepping-Stones Introgression

The hybridization and introgression between wild plants is a very well known phenomenon. Ellstrand et al. [2] estimated that there are more than 1000 well studied and published examples of spontaneous plant hybridization. Although at generally low frequencies and over long periods of time, genes (and thus also transgenes) can be spontaneously introgressed between different wild species [5]. It is therefore surprising that there are practically no detailed studies and exhaustive reviews on the importance of wild-to-wild hybridizations and wild-to-wild bridges in the context of the transgene flow and GE crops (Fig. 1).

How common are the natural hybridization processes between wild plant taxa? To answer this question we have to remember that there are two possible outcomes of hybridization [1]. The first outcome is the present ongoing introgression. Mallet [50] based on fundamental work on hybrid flora of the British Isles by Stace [51] estimated that at least 25% of all wild plant species are able to hybridize spontaneously and/or are involved in ongoing introgression processes with other wild species. Ellstrand et al. [52] using the same data set concluded that up to 34% of families and 16% of all genera in Great Britain have at least one reported hybrid. Additionally, there are many very well-studied genera with numerous closely related species producing hybrid swarms, such as *Salix* [53], *Quercus* [54] or *Eucalyptus* [55]. Rieseberg [56], based on the calculations of Ellstrand et al. [52], concluded that we could expect a worldwide total of 27 500 hybrid combinations among all Angiosperms. He added however, that it could be strongly underestimated since many regions, especially the tropics, are weakly explored and documented as far as their hybrid flora is concerned.

The second result of hybridization is the ancient and present speciation [56–58]. Indeed, in many families and genera, polyploidization and hybridization were the main mode of speciation and diversification. Ellstrand et al. [2] based on the summarizing works of Grant [59] and Arnold [60] concluded that more than 70% of plant species originated from hybrids.

As a consequence of these two well-documented hybridization outcomes, it has been often stated that the natural interspecific and even intergeneric hybrid formation is ubiquitous and uniform among higher plants [61, 62] or even the rule rather than the exception [63]. However, Ellstrand et al. [52] demonstrated clearly that the spontaneous hybridization is non-randomly distributed among systematic plant groups. By analyzing five biosystematic floras from Europe, North America and the Hawaiian Islands they showed that certain phylogenetic groups are predisposed for hybridization. To the most important hybrid families in practically all analyzed regions belong such crop-plant families as Poaceae, Asteraceae, Rosaceae and Fabaceae. Ellstrand et al. [2] enumerated 13 of the most important food crops grown for human consumption. Among them seven belong to Poaceae (*Eleusine, Hordeum, Oryza, Saccharum, Sorghum, Triticum, Zea*), and three to Fabaceae (*Arachis, Glycine, Phaseolus*). Hybridization seems therefore to be concentrated in a relatively restricted fraction of families and/or genera. Moreover, many members of these highly hybridizing families have been genetically modified and mainly possess numerous wild relatives. Table 3 lists all major plant genera and families of European flora containing crop plants with reported genetic transformation and/or with GE species used for field trials. It shows how many wild relatives of transformed crops could be found in Europe and which of those taxa possess the highest ability for complex hybridization. Here again the family of Poaceae has the most important potential for wild-to-wild bridge formation. Numerous of its mem-

Table 3 Number of wild relatives in European flora of the most important crop plants used for genetic transformation (only genera with GE members commercialized or used for field trials are listed) and estimation of their potential for wild-to-wild bridge formation. Symbols: – hybridization not observed, + hybridization observed but rare, ++ hybridization frequent, +++ hybridization extremely frequent (based on [51, 71], for *Lolium, Festuca* and *Poa* see also [112–160])

Genus	Nb. of wild (naturalized) species in Europe*	Potential for wild-to-wild bridge formation in Europe		
		intrageneric	intergeneric	hybridizing with
***Poaceae*:**				
Agrostis	24 (1)	+++	++	*Polypogon, Calamagrostis*
Avena	10 (2)	++	–	–
Cynodon	1	–	–	–
Festuca	165	+++	++	*Lolium, Vulpia*
Hordeum	8 (1)	++	++	*Agropyron, Elymus*
Lolium	5	+++	++	*Festuca*
Poa	43 (1)	+++	–	–
Triticum	3	+++	+++	*Aegilops, Elymus, Secale*
Zea	0	–	–	–
***Rosaceae*:**				
Fragaria	4 (1)	+	–	–
Malus	6	++	++	*Pyrus, Sorbus*
Prunus	19 (2)	++	–	–
Pyrus	11	++	++	*Malus, Sorbus*
Rubus	c. 75 (c. 3)	+++	–	–
Asteraceae:				
Cichorium	3	–	–	–
Helianthus	3 (7)	+	–	–
Lactuca	15	++	–	–
***Fabaceae*:**				
Glycine	0	–	–	–
Medicago	35 (2)	+	–	–
Pisum	1	–	–	–
***Solanaceae*:**				
Lycopersicon	0	–	–	–
Nicotiana	3(4)	–	–	–
Solanum	3 (9)	+	–	–
Other families:				
Beta	5	+	–	–
Brassica	20	+++	+++	*Raphanus, Sinapis, Diplotaxis, Hirschfeldia, Eruca, Erucastrum*
Cucumis	(1)	–	–	–
Cucurbita	0	–	–	–

Table 3 (continued)

Genus	Nb. of wild (naturalized) species in Europe*	Potential for wild-to-wild bridge formation in Europe		
		intrageneric	intergeneric	hybridizing with
Daucus	10	+	–	–
Picea	2 (c. 8)	–	–	–
Pinus	13 (c. 13)	–	–	–
Populus	4 (c. 6)	+++	–	–
Vitis	1 (c. 9)	+	–	–

* wild: native species including cultivated species capable of formation of weedy subspontaneous populations; naturalized: non-native species naturalized in Europe.

bers form easily both intra- and intergeneric hybrids. The only groups which could be compared with Poaceae are some genera of the family Rosaceae (mainly fruit trees) and the very well-known *Brassica* coenospecies complex.

It is worth mentioning that detailed studies and surveys on natural hybridization in wild taxa are extremely difficult. Abbott [64] and Ellstrand et al. [52] pointed out that the main limitation is the scarcity of modern biosystematic floras containing complete ecological, evolutionary and genetic information needed for such surveys. Additionally, the documentation of hybridization and introgression faces several methodological and theoretical difficulties. There are many methods used in identifying hybrids ranging from relatively simple morphological measurements to complex molecular and phylogenetic analyses. However, the majority of them, if not all, suffer from the fact that there are multiple explanations for the morphological and/or molecular intermediacy of a given hybrid candidate taxon [57]. The morphological similarity for example could be simply a result of convergent evolution. Martinsen et al. [26] concluded that the hybrid detection based on morphological characteristics is additionally constrained by backcrosses, since it is known that a backcrossed hybrid often resembles the parental species. The development of molecular genetic markers has facilitated studies of hybridization and allowed one to detect even very low levels of introgression. Additionally, it is possible with the molecular markers to track both the nuclear and cytoplasmic gene flow. However, the presence in one individual of molecular markers from two different species could be explained not only through recent hybridization but also due to shared ancestral characters (symplesiomorphy [26, 57]). The differentiation between contemporary versus ancient introgression is difficult and has been studied in only a few taxa [26, 65].

3.3
Poaceae: Example of a Biologically Predisposed Family for Wild-to-Wild Bridge Formation

Table 3 and detailed comparative studies mentioned above [2, 51, 52] demonstrate clearly the enormous potential of the family *Poaceae* for the wild-wild hybridization processes. The importance of the *Poaceae* as an object of research reflects their ecological and biogeographical success as well as their enormous economic value [66]. They occupy almost every habitat around the world, often being the dominating organisms [67]. The *Poaceae* comprises about 10 000 species and between 600 and 900 genera [68, 69]. In addition to that, the family contains a very high percentage of species and cytotypes of polyploidy origin. More than 80% of grass species have undergone polyploidy which represents the highest percentage in Angiosperms. Such a high level can be explained by successive regressions and extensions of the ranges which would favour secondary contact zones between related taxa, their hybridization and their subsequent polyploidization [70].

According to Wipff [71] one of the most important grass groups being currently used in genetic transformations are the forage grasses as well as grass species used for turf and erosion control. Furthermore, Wipff gives four main reasons why this group is particularly at risk of spreading transgenes: (1) they have undergone relatively little domestication; (2) they have usually numerous wild relatives; (3) they grow often in sympatry with these; (4) they can grow as weeds outside cultivated areas or in other crop cultures. To this grass group belong such common and species-rich European and North American genera as *Lolium, Festuca, Poa* and *Agrostis*. In the United States not less than 187 field tests were carried out between 1993 and 2006 with transgenic *Agrostis stolonifera*, 36 with *Poa pratensis*, 26 with *Festuca arundinacea*, 17 with *Cynodon dactylon* and 6 with *Lolium perenne* [72]. All mentioned species possess numerous wild relatives in Europe (Table 3) and are capable of hybridizing easily with them (e.g. *Festuca* with ca. 165 species, numerous subspecies and swarms of hybrids in Europe).

Figure 2 gives an example of intrageneric wild-to-wild hybrid complexes in *Poa*. Genus *Poa* contains approximately 43 species in Europe (Table 3) and 300 species worldwide. Intergeneric hybridization is extremely common and results in serious classification difficulties [71, 73]. In *Poa pratensis*, which absorbed genomes from many different taxa, it is even impossible to trace its ancestors [71, 74]. It was shown additionally that F1 hybrids between different *Poa* species can be completely fertile [75]. Figure 2 shows that almost 1/3 of all European *Poa* species are able to hybridize. They have mainly sympatric distribution even at a local level and have similar phenology. It is additionally very probable that more detailed studies would reveal much higher levels of intrageneric hybridization between members of the genus *Poa*.

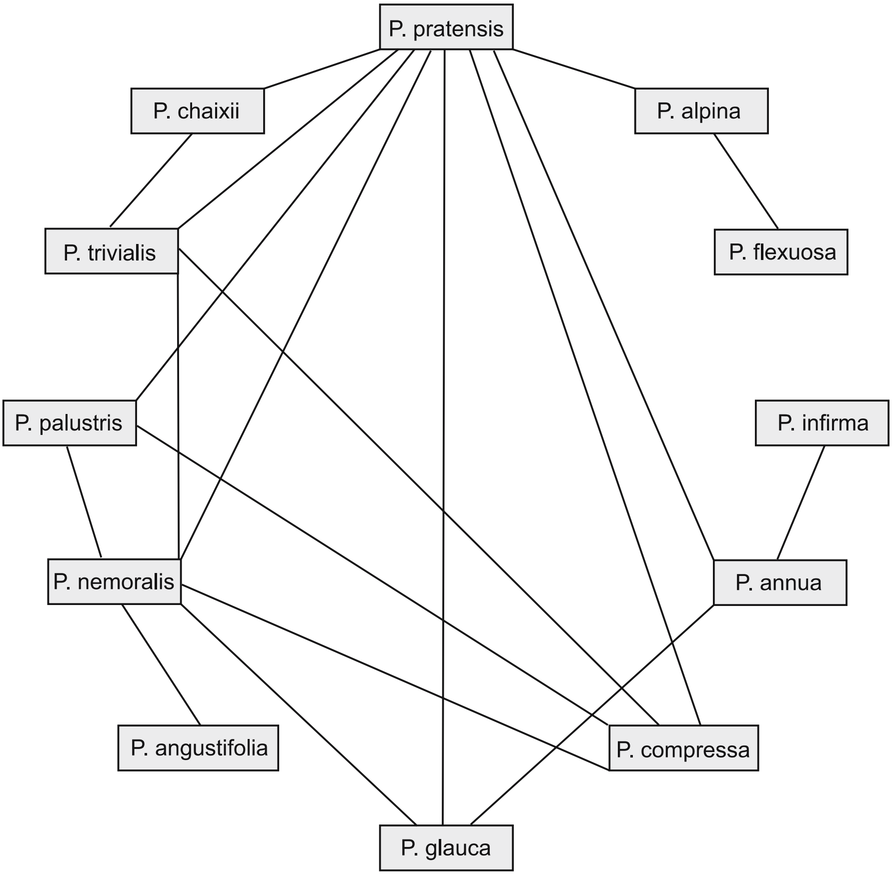

Fig. 2 Wild-to-wild bridges: possible intrageneric hybridization in the grass genus *Poa* [112–160]

Figure 3 gives some further examples of wild-to-wild inter- and intrageneric hybrid complexes in three common European genera of *Poaceae*. The reproductive compatibility and hybrid viability (even at intrageneric level) between *Lolium, Festuca* and *Vulpia* are very well documented (e.g. [51, 76–78]). Intrageneric spontaneous hybrids between *Festuca* and *Lolium* (= x *Festulolium*) are not rare (see also Table 3), they can be fertile and have an ability to backcross with either of the parents [71, 79]. The commonest x *Festulolium* in Europe is the hybrid between *F. pratensis* x *L. perenne* (= x *Festulolium loliaceum*) which can be found in different types of pastures and meadows from Norway to Italy [51, 80, 81]. Figure 3 shows additionally that there are certain species complexes where hybrid combinations are possible in all directions. This is the case for example in the following five species: *Festuca pratensis, F. arundinacea*, *F.*

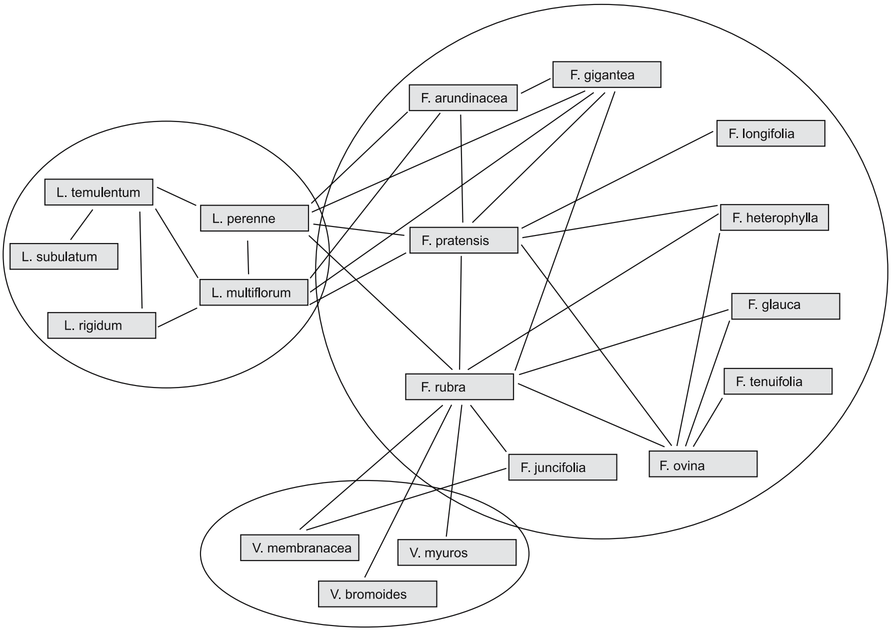

Fig. 3 Wild-to-wild bridges: possible intra- and intergeneric hybridization between selected grass genera: *Lolium*, *Festuca* and *Vulpia* [112–160]

gigantea, *Lolium perenne* and *L. multiflorum*. The close relation of these species could be also demonstrated experimentally [82]. Several authors proposed even to join both genera or to move some *Festuca* species into genus *Lolium* [83, 84]. Additionally, some studies on the chromosome structure of *F. pratensis*, *L. perenne* and *L. multiflorum*, concluded that there are practically no barriers for gene exchange between these species [71, 78].

The majority of species represented in Figs. 2 and 3 fit very well the general characteristic of taxa predisposed for hybridization [12, 52]. They are outcrossing with incomplete reproductive isolation between species, they are mainly perennials with well-developed vegetative spread. Further, they are wind-pollinated and the pollen dispersal up to 21 km has been shown (e.g. for *Agrostis* [16]). Thus, the geographic proximity as well as pollination does not represent any constrains. Additionally, they flower over a very long time period from May till August, thus even at a local scale the phenological overlapping is very common.

The examples described above illustrate clearly that in selected vascular plant families and genera we could potentially expect a stepping-stone spread and exchange of genes with unpredictable effect. Absolute containment of transgenes will be in such taxa practically impossible. Therefore, more experimental and descriptive work has to be done in order to evaluate the existence and importance of wild-to-wild bridges among a spectrum of taxonomic plant groups as broad as possible.

4
Genetic and Ecological Consequences on Wild Relatives

4.1
Genetic and Ecological Consequences of Outcrossing

Outcrossing in plants may have different impacts, depending on the relatedness of the taxa. When a single species is involved, chromosomes are homologous and pair regularly. On the contrary, when related taxa hybridize, recombinations occur between homeologous chromosomes with the possible consequence of irregular pairing, leading to unbalanced gametes with reduced fertility.

Hybridization between crops and wild relatives is a very ancient phenomenon which has been investigated for a long period from an agronomist point of view, as gene flow from the wild species to the crop might lead to reduced yield and loss of the genetic purity of the cultivated varieties. More recently, while GE plants have been developed, agronomists and ecologists have been concerned by the consequences of transgene escape into non-transgenic crop fields or in wild relatives.

Hybridization has genetic and ecological consequences. Genetic consequences may be defined as the effects of the insertion of the genes in the target species itself and on the expression of genes. On the other hand, ecological consequences are considered here as direct or indirect effects on fitness. We discuss below the two types of consequences separately.

4.2
Consequences of the Transgene

The transgene itself may have genetic consequences for the recipient plants by interacting with other genes and leading to untargeted effects. In order to investigate this aspect, among many others, *Arabidopsis thaliana* has been used as a model species. Metzdorff et al. [85] analyzed, using cDNA microarrays, six independently transformed *A. thaliana* lines characterized by modified flavonoid biosynthesis. Although these transgenic lines possessed different types of integration events, no unintended effects were identified.

Genetic transformation could also affect fitness, and may be in this case associated with a physiological cost. For example, significant reduction of fitness was observed repeatedly associated with resistance to herbicide. Bergelson et al. [86] observed for *A. thaliana* a 34% reduction in seed production for a mutant acetolactase synthetase gene that confers resistance to the herbicide chlorsulfuron, in comparison to the non-transgenic lines. This cost in fitness was caused by pleiotropic effects due to the presence of the resistance genes itself, while no cost was associated with the expression of kanamycin resistance. Purrington and Bergelson [87] obtained similar results by comparing mutant and transgenic herbicide resistant lines in two different environmental conditions: with or without fertilizer treatments. The cost of resistance appeared in both treatments for the transgenic line, while no cost was associated with the mutant line in the high fertilizer treatment. Other untargeted effects may appear, such as the change in outcrossing rate observed in an outdoor experiment involving transgenic *A. thaliana*, without the proof that it was caused by the transgene itself [88].

Transgenesis may have also unexpected effects on crops. For example, the lignin content of Bt corn was significantly higher than that of non-Bt corn [89]. A change in lignin content might affect the action of herbivores and have ecological consequences.

Moreover, Prescott et al. [90] demonstrated that post-translational modification of a plant protein (α-amylase inhibitor-1 from the common bean (*Phaseolus vulgaris*)) led to the synthesis of a structurally modified form of the protein in pea (*Pisum sativum*). This protein showed altered antigenic properties. While this example concerns human health, it shows that untargeted effects of transgenic plants on protein expressions occur. Consequently, we can infer that similar effects could lead to changes in ecological properties.

4.3
Consequences for Wild Relatives

Introgression involves chromosome segments containing possibly several genes. Therefore, the consequences of introgression will depend on the genes included in the introgressed segment, and on the site of introgression in the recipient species (linkage to other crop genes, pleiotropy). Similarly, introgression from a transgenic crop to a wild relative will also depend on the insertion site of the transgenic line. Genetic consequences are expected to be those of conventional lines, except for the effect of the transgene itself.

4.3.1
Inference from Natural Observations

Natural hybridization is frequent in nature and the fitness of hybrids may be lower, equal or higher than that of their parents [91]. Hybrid inferiority has been recognized as a rule for a long time. More recently, the importance of hybridization for evolution and speciation has emerged (e.g. [2, 59, 60]). Hybrids may be at the origin to new lineages, which may lead to new species. Speciation might be either progressive or abrupt, when polyploids are formed by chromosome doubling. Because of their different chromosome number, allopolyploids are reproductively isolated, at least partly, from their parents.

According to Burke and Arnold [92], different genetic mechanisms operate behind low and high fitness of the hybrids. Hybrid inferiority would be caused in most cases by negative epistasis, while heterosis would be mostly the consequences of the segregation of additive genetic factors.

4.3.2
Inference from Conventional (Non-Transgenic) Wild x Crop Hybrids

Hybridization followed by repetitive backcrosses lead to introgression, the transfer of a part of the genome of one species to another. Depending on the introgressed genes and on their expression, introgression may lead to new characteristics which could affect the ecological properties of the target species. Experimental data produced a broad spectrum of results on the relative fitness between hybrids and their parents.

The effectiveness of gene flow will depend on the viability and the fertility of hybrids and of subsequent backcrosses. Several studies have involved hybrids between a conventional crop and their wild relatives. For example, Hauser et al. [93, 94] have investigated the fitness of F1 hybrids, as well as F2 hybrids and backcrosses between *Brassica rapa* and oilseed rape (*B. napus*) in experimental crosses. Hybrids were as viable as their parents, produced more pods, but these later contained fewer seeds, with an overall fitness that was intermediary to their parents [93]. The fitness of F2 and backcrosses were on average lower, compared to that of their parents, and varied considerably, including individuals as fit as *B. rapa* [94].

In another study, interspecific F1 hybrids between wild and cultivated radishes (*Raphanus raphanistrum* x *R. sativus*) had a lower fitness than the wild plant. Nevertheless, a field experiment was set up with one half containing F1 wild-crop hybrids and the other half wild. After three years, the dominant white color of the flower of the crop persisted at a frequency ranging from 8% to 22% [95]. A similar study on carrot (*Daucus carota*) showed that hybrids between cultivated and wild carrots were more sensitive to frost than the wild parents, which limited their survival [96].

Weed x crop hybrids between *Sorghum halepense* (Johnsongrass) and *S. bicolor* (cultivated sorghum) did not show any difference in fitness, suggesting that in this case, no barriers to gene flow exist [97]. Contrasting with that, a second generation of hybrids (S1 and BC1) between *Lactuca serriola* (prickly lettuce) and *L. sativa* (cultivated lettuce) germinated and survived better than their wild relative. Seed output of both classes of hybrids was greater with *L. sativa* but no significant difference was found with *L. serriola* [98].

4.3.3
Inference from Transgenic Wild x Crop Hybrids

Table 4 summarizes the results of fitness measurements comparing the hybrids of different generations for five crops, partly derived from Hails and Morley [1]. Non-transgenic hybrids have usually lower, equivalent or intermediary fitness then their parents.

A notable exception is the experiment of Guadagnuolo et al. [11] which demonstrated heterosis of hybrids between glyphosate-tolerant maize (*Zea mays*) and teosinte (*Z. mays* ssp. *mexicana*), when compared to the wild parent. Nevertheless, in the absence of selection pressure with herbicide, no difference was detected between transgenic and non-transgenic hybrids.

Sunflowers have been extensively studied. Burke and Rieseberg [10] investigated transgenic sunflower with an inserted gene of oxalate oxidase (OxOx) conferring enhanced white mold resistance in cultivated sunflower. They backcrossed it with wild sunflower. No cost of the transgene was observed in the absence of the pathogen. When the plants were infected, the transgene decreased the probability of infection, this later having a negative effect on seed output. Moreover, the disease effect varied among locations and no generalization was possible. The authors insisted on the necessity of replicating the experiment over space and time, as well as on the importance of genetic background and of environmental conditions.

Snow et al. [99] demonstrated that, in the field, male sterile wild sunflowers introgressed with a Bt transgene produced more inflorescences than those without the transgene. These advantages were related to a decrease in insect damage. Greenhouse experiments did not reveal any fitness cost of the transgene.

In hybrids with transgenic rapeseed, relative fitness varied considerably, depending on the transgene and on the presence of associated selective pressure by insect herbivores. Transgenic hybrids between wild *Brassica rapa* and rapeseed possessing a Bt transgene performed better than non-transgenic hybrids in the presence of herbivores, while their fitness was lower when herbivores were absent, showing a physiological cost of the transgene [100]. On the contrary, performances were equivalent in hybrids with or without a transgene coding for high laurate content [101, 102]. The same was true for glufosinate tolerance, in the absence of herbicide treatment.

For practical reasons, only one transgenic line was used in most of the cited experiments, instead of using so-called "sister lines", possessing the same transgenic construct but in different insertion sites. It is then delicate to assess the effect of the transgenes themselves on the fitness of hybrids and subsequent backcrosses, because the consequences on fitness may not be due necessarily to the transgene, but could depend on its insertion site. Moreover, environmental conditions and the density of plants may also influence relative fitness [10, 103].

Table 4 Comparisons of fitness of F1 and BCs for some crops; data compiled partly from [1]

		NT (non transgenic), T (transgenic)	Type of transgene	Hybrid generation	Fitness (E=equivalent, H=higher, I=intermediary, L= Lower)	Refs.
Oilseed rape						
Brassica napus	*Brassica rapa*	NT		F1	I	[93]
B. napus	*B. rapa*	NT		F2 and BC1	L	[94]
B. napus	*B. rapa*	T	glufosinate tolerance	F1	female fitness L to H: frequency and density dependent, male fitness L	[103]
B. napus	*B. rapa*	T	Bt	F1	H, in presence of herbivores; L in absence	[100]
B. napus	*B. rapa*	T	Bt	BC2	E (low herbivory)	[105]
B. napus	*B. rapa*	T	high laurate	F1	E	[101]
B. napus	*B. rapa*	T	high laurate	F1	E	[102]
B. napus	*B. rapa*	T	glufosinate tolerance	BC3	E	[106]
B. napus	*B. rapa*	T	glufosinate tolerance	BC1	E	[107]
Sunflowers						
Helianthus annuus	*H. annuus* (wild type)	NT		F1	L	[108]
H. annuus	*H. annuus* (wild type)	T	Bt transgene	BC1	H (natural herbivory)	[99]
H. annuus	*H. annuus* (wild type)	T	white mould resistance	BC3	H to E	[10]

Table 4 (continued)

		NT (non transgenic), T (transgenic)	Type of transgene	Hybrid generation	Fitness (E=equivalent, H=higher, I=intermediary, L= Lower)	Refs.
Sugar beet						
Beta vulgaris ssp. *vulgaris*	*B. vulgaris* ssp. *maritima*	NT		F1, F2	E	[109]
B. vulgaris ssp. *vulgaris*	*B. vulgaris* ssp. *maritima*	T	viral resistance	F1, F2	E	[109]
Squash						
Cucurbita pepo	*Cucurbita pepo* (wild type)	NT		F1, F2 and BC1	L (F1) to E (F2 and BC1)	[110]
C. pepo	*C. pepo* (wild type)	T	viral resistance	F1, BC1 and BC2	L (F1) to H (BC1 and BC2) in case of high disease pressure. L for all in case of low disease pressure	[110]
C. pepo	*C. pepo* (wild type)	T	resistant to two pathogenic viruses	F1	L (survival and seed production)	[111]
Maize						
Zea mays	*Z. mays* ssp. *mexicana*	NT		F1	H then wild species	[11]
Zea mays	*Z. mays* ssp. *mexicana*	T	glyphosate tolerance	F1	H then wild species	[11]

Summarizing, costs of the transgene have been observed in some cases but not in all. On the other hand, it is worth noting that in the mentioned studies, positive effects on fitness of transgenic hybrids has been interpreted as the direct effect of the transgene itself.

5
Conclusion

To date, a considerable amount of data has been gathered on potential and actual gene flow between crops and wild relatives. Such knowledge is extremely useful for the risk assessment associated with GE crops, especially when considering the regional component of the floristic composition. The investigation of the case of Switzerland reveals differences with Europe, which modulate the evaluation of risk for several crops. It reveals also that not only the presence of wild relatives differ geographically, but that their genetic composition may vary and strongly influence gene flow, as was illustrated for alfalfa [40].

Other issues merit further investigation. Indeed, transgenes can be transmitted from crops to the closest wild relatives, but can also migrate further to other species, by successive crosses. A bibliographical survey of Poaceae illustrate that hybridization is widespread in some taxonomical groups.

Genetic and ecological consequences of the transgene in a wild species have also been poorly investigated up to now. The few existing studies show different pictures according to the species and the inserted trait. More investigations are needed to dissociate the importance of the insertion site and of the transgenes themselves. Moreover, the fitness of hybrids may vary according to environmental conditions and these interactions merit evaluation in nature. It is interesting to note that several examples demonstrate that transgene expression give advantages to the wild species in cases of selective pressure, as for the Bt gene in the presence of herbivores. While generalization is difficult, any type of transgene which would influence fitness positively, such as for example the resistance to diseases could confer to the wild species a real ecological advantage.

Finally, given the diversity of the results observed in the various studies on gene flow, and on its consequences, it seems almost impossible to address all the questions experimentally. On the contrary, it is probably necessary to produce enough empirical data, in order to build realistic and reliable predictive models.

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