

Review

Crop-to-wild gene flow, introgression and possible fitness effects of transgenes

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Crop-to-wild gene flow has received close attention over the past ten years in connection with the development and cultivation of transgenic crops. In this paper, we review key examples of crop/wild sympatry and overlapping flowering phenology, pollen and seed dispersal, the barriers to hybridisation and introgression, the evolution and fate of interspecific hybrids, their fitness, and the potential cost of transgenes. We pay particular attention to ways in which the evolution and divergence between crops and their wild relatives may interfere with these successive steps. Our review suggests that crop-to-weed gene flow is highly idiosyncratic and that crop gene dispersion will certainly be very difficult to preclude totally. Future directions for research should thus focus on the long-term establishment and effects of transgenes on natural communities.

Keywords: gene flow / hybridization / introgression / hybrid fitness / transgene cost / crop-wild complexes

INTRODUCTION

Gene exchange has always been a major determinant of the evolution in crop-weed-wild complexes, *i.e.* groups of crops, accompanying weeds and wild species that exchange genes. On the one hand, many crops have evolved through recurrent cycles of hybridization/differentiation from their wild/weedy relatives (Harlan, 1992). Wild species are still used in plant breeding as a rich source of genes that may be transferred into crop germplasm in order to expand its genetic basis, to confer resistance or increase tolerance to biotic and abiotic stresses, or to transfer new agronomic features into the cultivated forms (Frankel et al., 1995). On the other hand, natural crop-to-wild hybridisation has sometimes led to the extinction of crop relatives or has generated new races of weeds that are more aggressive and better adapted to man-made habitats (Ellstrand et al., 1999). Despite its obvious evolutionary significance, crop-to-weed gene flow has received little experimental attention.

The prospect of commercial release of genetically engineered plants has begun to reverse this trend. A large

literature is now dedicated to the assessment of transgene escape through pollen and seeds, and its potential ecological and agricultural effects (Snow and Morán Palma, 1997). The main concern is that widespread cultivation of some transgenic crops could accelerate the evolution of undesirable and more invasive weeds, thereby leading to the erosion of biodiversity or to ecosystem disequilibrium effects (Tiedje et al., 1989; Snow and Morán Palma, 1997). Although this threat applies to traditionally bred crops (Ellstrand et al., 1999), differences between genetic engineering and traditional breeding regarding the nature, number, and effects of the introduced traits may make some types of GMOs (Genetically Modified Organisms) more risky than traditional crops (Regal, 1994). Other potential problems due to the cultivation of GMOs have been identified. Crop-to-crop gene flow may lead to gene stacking (Ellstrand, 2001), crop product pollution (while crop diversification is ongoing), the rapid modification of pest communities and selection of more aggressive

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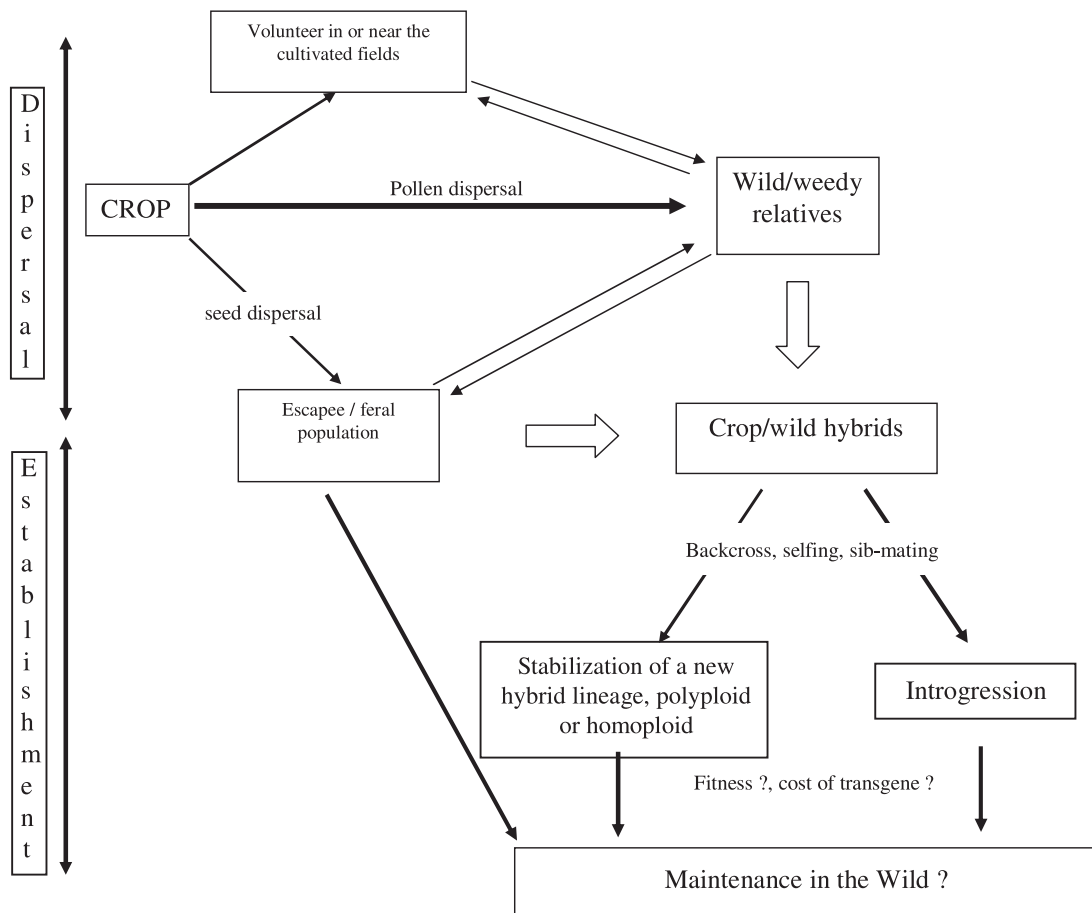


Figure 1. Schematic representation of the main steps for crop-to-wild gene flow.

pathogens, and unintended effects on non-target organisms (Poppy, 2000). Notwithstanding their importance, we will not address these questions in this paper, but focus on the issues related to crop-to-wild gene flow.

Effective crop-to-wild gene flow depends on the completion of a number of successive steps required for gene dispersal (Fig. 1): (i) the presence of crops and close relatives growing nearby, (ii) their biology and phenology in relation to pollen and seed dispersal, (iii) the production of viable and fertile F1 hybrids, (iv) the production of fertile successive generations, (v) the opportunity for gene transmission, chromosome recombination and crop gene introgression into the wild genetic background, and ultimately (vi) the persistence of introgressed crop genes in natural communities. In this paper, we will examine key examples for each of these, and point out how the evolution of both crops and

their wild relatives affects the potential for crop-wild hybridization and introgression.

(TRANS)GENE DISPERSAL

Spatial gene dispersal *via* pollen: the role of the cultivated “source” and natural “sink” populations

Crop-to-wild gene flow has long been considered to be mostly pollen mediated (Ellstrand and Hoffman, 1990). Gene dispersal through pollen requires the presence of sexually compatible relatives in the regions where a crop is commonly grown. On a large spatial scale, the probability of crop gene flow depends on the geographic distributions of crops and their wild relatives; such contacts are generally more frequent in the crop’s centers of origin and/or centers of diversity (Simmonds, 1995).

On a smaller spatial scale, crop gene dispersal is expected to be stronger into its more common weedy relatives, because there will be closer and more numerous zones of co-occurrence. These species are also to be considered as the most risky potential recipients for transgenes because they are already adapted to man-made habitats and can be difficult to control.

Several authors have listed the major crops and their co-occurring wild relatives in the following western countries: the Netherlands (De Vries et al., 1992), UK (Raybould and Gray, 1993), USA (Snow and Morán Palma, 1997), Austria (Pascher and Gollmann, 1997). These surveys are important to identify the most likely wild partner(s) for crop-to-wild gene flow and set up research priorities. However, even if broad information on the distribution and importance of wild species is available, little is known about the precise extent of their proximity to related crops. Remote sensing has recently been used to address this problem. For example, satellite imagery proved to be an efficient method for identifying all the possible contact zones between *Brassica napus* and weedy *B. rapa* across south-east England (Wilkinson et al., 2000).

The potential for spontaneous crop-to-wild gene flow is then determined by the extent to which flowering period overlaps and by pollen dispersion. Experimental data on comparative crop/wild phenology are scarce, but suggest that wild populations usually display a wider range of flowering dates than crops. This difference in the length of flowering makes the overlap more likely, because at least some wild plants will flower in synchrony with most of the cultivated ones. Conversely, overlaps can be limited, so that many wild plants will be isolated from the cultivated pollen source (Renno and Winkel, 1996). Growing early or late cultivars may thus have significant effects on the extent of crop gene dispersal.

As regards crop pollen dispersal, most studies have described highly leptokurtic distributions, with most pollen grains moving over very short distances, and then more scarcely over longer distance. These observations apply to both wind- and insect-pollinated species: turnip (Manasse, 1992), cotton (Karieva et al., 1994), potato (Tynan et al., 1990; McPartlan and Dale, 1994; Skogsmyr, 1994; Conner and Dale, 1996), oilseed rape (Scheffler et al., 1993; Morris et al., 1994; Lavigne et al., 1998), radish (Klinger et al., 1992), ryegrass (Giddings et al., 1997a), sunflower (Arias and Rieseberg, 1994), meadow fescue (Rognli et al., 2000), and alfalfa (St Amand et al., 2000). Although long-range pollen movement is a low probability event (Karieva et al.,

1994), hybridization between crops and weed/wild relatives have also been frequently reported up to hundreds of meters away from the cultivated source (Kirkpatrick and Wilson, 1988; Klinger et al., 1992; Arias and Rieseberg, 1994; Arriola and Ellstrand, 1996). Low-frequency long-distance dispersal is highly relevant for risk assessment, since a small number of successful migrants per generation is sufficient to modify the structure of recipient populations.

Generalizations about the expected distribution of hybridization events are not straightforward. Many studies have demonstrated considerable, and almost unpredictable, variations of crop-to-weed gene flow between discontinuous patches of plants (Raybould and Gray, 1993; Morris et al., 1994; Giddings et al., 1997b). These variations are not always related to interpatch distance (Manasse, 1992) and depend on many extrinsic factors (Raybould and Gray, 1993; Giddings et al., 1997b). Notably, the shape, relative size and density of both the crop “donor” and “recipient” wild/weedy populations prove to be important (Jorgensen and Andersen, 1994; Chèvre et al., 2000). For instance, Ellstrand et al. (1989) found essentially no gene exchange among three small populations of insect-pollinated wild radish a few thousand meters apart, but substantial gene flow into them from very large populations thousands of meters away (see also Klinger et al., 1992). Such asymmetric gene flow from cultivated fields into wild/weedy populations is expected when the relative size of the recipient wild/weedy populations is small (Ellstrand, 1992; Ellstrand et al., 1999) or when they are more fragmented.

Modelling pollen dispersal offers a potential solution to address these problems, provided that the pollen distribution of each individual plant is adequately known (Lavigne et al., 1998). This approach requires (i) developing robust estimators of individual pollen dispersal functions (which fit experimental data) and (ii) using these functions to achieve quantitative predictions for pollen dispersion in various agricultural designs.

Finally, it must be acknowledged that crop gene dispersal does not necessarily imply that gene flow occurs only in one direction, from crops into their wild relatives. Using cytoplasmic DNA markers, Boudry et al. (1993) demonstrated that weed beets arise from the accidental pollination of cultivated sugar beets (*Beta vulgaris* ssp. *vulgaris*) by adventitious wild beets (*B. vulgaris* ssp. *maritima*) in seed production areas. Such wild-to-crop gene flow can be expected when crop seeds are able to disperse and establish feral populations.

Spatial and temporal (trans)gene dispersal via seeds and vegetative propagules

Two different situations should be considered when assessing crop gene dispersal *via* seeds or vegetative propagules. First, dispersal may occur in space, due to the movement of seeds or vegetative propagules from cultivated fields into natural communities. This first route may result in the formation of feral populations when escaped crop plants are able to survive in the absence of human care (*e.g.* forage crops). Second, dispersion may occur in time due to delayed hybridization between wild and cultivated plants that have moved into natural communities (Linder and Schmitt, 1994; 1995) or persisted after harvesting.

Little is known about the extent of spatial seed dispersal (Crawley and Brown, 1995) or the potential persistence of feral populations (Crawley et al., 1993; Pessel et al., 2001). Spatial seed dispersal by natural means is often considered to be low because most crops have lost their ability for independent seed dispersal (De Wet and Harlan, 1975). Although substantial dispersion can be mediated by seed spillage during harvest (Price et al., 1996) or transport (Crawley and Brown, 1995) resulting in dispersal over very long distances, crop seed dispersal is expected to be over shorter distances than pollen. The potential for temporal dispersal of transgenes primarily depends upon the initial level of crop seed dormancy, the longevity of seeds in the soil, and the germination behavior of seeds in response to environmental cues (Linder and Schmitt, 1994). Although most crops lack extensive seed dormancy and longevity (Lewis, 1973; Hails et al., 1997; Chadoeuf et al., 1998), some of them, such as oilseed rape, retain sufficient dormancy and longevity to persist as volunteers during crop rotation, notably when huge amounts of seeds are lost at harvest. Feral populations of oilseed rape have persisted along roadsides for 3–4 years after dispersal (Crawley and Brown, 1995) or even longer (Pessel et al., 2001). The dynamics of these feral populations in natural or semi-natural habitats is complex. Their persistence may be longer if transgene(s) increase the fitness of the escaped plants (Stewart et al., 1997).

Rare establishment of crop individuals *via* seeds or vegetative propagules can provide repeated opportunities for the production of advanced generations of hybrids, and increase the likelihood of introgression over evolutionary time (*e.g.* Hodges et al., 1996). Crop seed dispersal can therefore be an important step for further gene flow between wild and cultivated populations. This

contention calls for a better characterisation of spatial and temporal crop seed dispersal and persistence.

The production of F1 hybrids between crops and their wild relatives: insights into the nature and strength of reproductive barriers

The degree of cross-compatibility between cultivated plants and their wild relatives has received close attention (Tab. 1), primarily because crosses between crops and related species have been extensively used in plant breeding. More recently, the chance for reciprocal crop-to-wild hybridization has been evaluated for a large number of crops and their wild relatives: for example, *Beta* (Santoni and Bervillé, 1992; Boudry et al., 1993), *Brassica* (Chèvre et al., 1998a), *Chenopodium* (Wilson and Manhart, 1993), *Cucurbita* (Kirkpatrick and Wilson, 1988; Wilson, 1990), *Helianthus* (Arias and Rieseberg, 1994; Whitton et al., 1997), *Medicago* (Jenczewski et al., 1999), *Oryza* (Langevin et al., 1990), *Pennisetum* (Robert et al., 1991), *Raphanus* (Klinger et al., 1992), *Setaria* (Till-Bottraud et al., 1992), *Sorghum* (Arriola and Ellstrand, 1996), *Triticum* (Seefeldt et al., 1998; Zemetra et al., 1998), and *Zea* (Doebley, 1990). These data indicate that crops are usually cross-compatible with their direct progenitors, and that the chance for crop-wild hybridization decreases with increasing genetic and phenotypic divergence (Tab. 1); a few intergeneric hybrids were also reported between some crops and wild related species (Tab. 1). In some instances, the level of crop-wild compatibility is genotypically determined (Baranger et al., 1995; Sacks et al., 1997), and under a simple genetic control. For example, a single locus restricts crossability between teosinte and maize (Evans and Kermicle, 2001).

Evidence that hybridization occurs between a large number of crops and their wild relatives suggests that most of the reproductive barriers that have accompanied the process of plant domestication (Harlan, 1992; van Raamsdonk, 1995) are imperfect. Domestication is a very recent process on the evolutionary scale; most crops and their progenitors have diverged for less than 12 000 years (Simmonds, 1995), which is not sufficient to establish impermeable reproductive barriers. For instance, neither mating systems nor polyploidy, which have been viewed as the most efficient mechanisms by which crops and their wild relatives can become reproductively isolated, ensure complete reproductive isolation.

Although most cases of crop-wild hybridisation are observed between outcrossing species (Kirkpatrick and Wilson, 1988; Doebley, 1990; Wilson, 1990; Klinger

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Table 1. Examples of the degree of cross-compatibility between crops and their wild relatives (data compiled from Gallais and Bannerot, 1992). For each cultivated species, the status of the ancestral species is first indicated (same species/sub-species/related species).

Cultivated species	Ancestral taxa					Other related taxa		
	Autogamous	Polyploid	Same species	Sub-species	Related species	Sub-species	Related species	Other genera
<i>Triticum turgidum durum</i> (durum wheat)	yes	yes	++			+ [1]	+ [1]	+/-
<i>Triticum aestivum</i> (bread wheat)	yes	yes	++			+ [1]	+ [1]	+/-
<i>Brassica napus</i> (oilseed rape)	mixed	yes	?	?	?	/	+ [1]	?
<i>Glycine max</i> (soybean)	yes	no			++ Glycine soja	/	-	?
<i>Hordeum vulgare</i> (barley)	yes	no			++ (<i>H. spontaneum</i>)	/	+	?
<i>Oryza sativa</i> L. (rice)	yes	no	++			++	+	?
<i>Pisum sativum</i> (pea)	yes	no		++		/	++	-
<i>Lupinus albus</i> (white lupin)	yes	no		++ <i>L. a. ssp. graecus</i>				
<i>Lycopersicon esculentum</i> (tomato)	yes	no	++			/	+	-
<i>Phaseolus vulgaris</i> (bean)	yes	no	++			/	+	-
<i>Dactylis glomerata</i> (dactyl)	no	yes	++			++	++	?
<i>Festuca arundinacea</i> (fescue)	no	yes	++			++	+	-
<i>Lolium perenne</i> (ryegrass)	no	yes	++			/	+	-
<i>Medicago sativa</i> (alfalfa)	no	yes	++			++	+ (perennial sp.)	
<i>Zea mays</i> (maize)	no	no		++ <i>Z.m. ssp. parviglumis</i>		++	++	+ (<i>Tripsacum</i> sp.)
<i>Helianthus annuus</i> (sunflower)	no	no	++			/	++	?
<i>Beta vulgaris</i> (beet)	no	no		++ <i>B.v. ssp. maritima</i>		/	+ [2] (sect. <i>Vulgares</i>)	-
<i>Trifolium pratense</i> (clover)	no	no	++			/	+ [2]	
<i>Capsicum annuum</i> (pimento)	no	no	++			/	+	-
<i>Brassica oleracea</i> (cabbage)	no	no	++			+	+ (chromosome doubling)	-
<i>Cynara scolymus</i> L. (artichoke)	no	no			++ (<i>Cynara cardunculus</i>)	/	+	-

++: Complete crossability; +: crossability with some reduced fertility of the hybrids; -: crosses impossible without artificial manipulations.

[1] Crosses are reported with sp. or ssp. that share at least one set of homologous chromosomes with the cultivated species.

[2] Crosses are limited to species belonging to the same section as the cultivated species.

/: No sub-species are known for the corresponding crops.

(?): Data not available.

et al., 1992; Boudry et al., 1993; Arias and Rieseberg, 1994; Whitton et al., 1997; Jenczewski et al., 1999), preferential self-fertilization in the wild or cultivated populations does not totally preclude efficient dispersion of crop genes (Robert et al., 1991; Wilson and Manhart, 1993; Arriola and Ellstrand, 1996; Seefeldt et al., 1998; Zemetra et al., 1998). Self-fertilization is rarely absolute. Most autogamous species show outcrossing rates varying from 0.5% up to 5–7%. Moreover, offspring between divergent autogamous species can demonstrate hybrid vigor that enhances the effective outcrossing rate of these species. Pollen competition can also account for the partial reproductive isolation between crops and relatives (Robert et al., 1991; Rieseberg et al., 1995) or change the frequency of interspecific hybridization. Hauser et al. (1997) showed that higher proportions of hybrid seeds were obtained on the diploid field mustard (*B. rapa*) and the allotetraploid oilseed rape (*B. napus*) when a mixture of pollen from the two species was applied.

Ploidy barriers in crop-wild complexes can arise when crops are domesticated after reaching a higher level of ploidy (e.g. *Triticum aestivum*, *Solanum tuberosum*, *Coffea arabica*, *B. napus*) and consequently these crop species lack wild relatives at the same level of ploidy. In other instances, diploid crops have polyploid relatives (e.g. *Sorghum bicolor* and *S. halepense*). In these complexes, the production of interspecific hybrids is reduced due to differences in ploidy levels between uniting gametes. However, interspecific hybrid production is possible and may be high when pollination occurs between an allopolyploid crop and one of its progenitors (Jorgensen and Andersen, 1994; Bing et al., 1996). Reciprocally, spontaneous hybridization between diploid *S. bicolor* and tetraploid *S. halepense* is frequent (Arriola and Ellstrand, 1996). Hybridization with less closely related species is also possible. It is generally more successful when the mother plant is the species with the higher ploidy level (Kerlan et al., 1992; Jorgensen and Andersen, 1994) because endosperm develops abnormally when the maternal:paternal genome ratio deviates from 2:1 (Johnston et al., 1980). The resulting triploid hybrids are not as systematically sterile as previously considered; some of them are able to produce euploid gametes and can thus serve as a bridges for gene flow or the formation of new polyploid species (Ramsey and Schemske, 1998). The production of unreduced gametes by the diploid parent can also contribute to the production of hybrids in the species with the highest ploidy level, since unreduced gametes restore the optimal 2:1 maternal:paternal genome ratio. The production of unreduced gametes is

usually variable in populations and depends on genotype, environment, and their interactions (Bretagnolle and Thompson, 1995). It has been shown to occur at up to 30% per plant per generation in certain species, and can therefore contribute to significant interploidy gene flow.

To conclude, a growing body of empirical data now demonstrates that crop-to-wild hybridization is likely when crops have sexually compatible relatives in adjacent ecosystems, although it often occurs at a very low frequency. Note that mating systems and differences in ploidy levels are not the only reproductive barriers that can limit crop-to-wild gene flow (see Arnold, 1997; Rieseberg, 1997; Rieseberg and Carney, 1998 for review). However, once gene dispersal has occurred, the question is then to understand whether genes will persist and establish in the natural populations of free-living relatives.

(TRANS)GENE ESTABLISHMENT IN NATURAL POPULATIONS

Until now, very few studies have attempted to measure directly the persistence of crop genetic markers in wild species after hybridization: e.g., raspberry (Luby and McNicol, 1995), sunflower (Whitton et al., 1997; Linder et al., 1998). For these two species, evidence of long-term introgression of crop genes into natural populations was reported (gene conferring spinelessness in raspberry, RAPD markers in sunflower). These direct measures of crop gene establishment raise questions about distinguishing introgressive markers from ones jointly inherited from a common ancestor. The same problem arises when population structure is used to infer past and present gene flow between crops and their relatives (Bartsch et al., 1999; Jenczewski et al., 1999). Most workers have thus concentrated on an alternative strategy that consists of analyzing the successive steps in the process of transgene establishment. Several determinants have been considered: (i) the genetic mechanisms that allow the transfer of cultivated genes into wild communities, (ii) the fitness of early hybrids relative to their parents, (iii) possible fitness costs or benefits that are associated with particular transgenes.

Evolution and fate of interspecific hybrids: genetic mechanisms of introgression and speciation

Introgression, the permanent incorporation of genes from one set of differentiated populations into another (Rieseberg and Wendel, 1993), is viewed as a common

outcome of hybridization. Although introgression between wild and domesticated plants is suspected to be widespread (De Wet and Harlan, 1975; Harlan, 1992; Rieseberg and Wendel, 1993; Ellstrand et al., 1999), it has rarely been demonstrated in wild populations (Luby and McNicol, 1995; Whitton et al., 1997; Linder et al., 1998). From a mechanistic standpoint, the extent of gene introgression depends on the interactions between recombination and selection.

At the whole genome scale, the probability for crop gene transfer depends on the level of genetic and structural homology between the genomes of crops and wild plants. More frequent introgression is expected when crops and their wild relatives share high levels of homology: e.g. *Beta vulgaris* × *B. maritima* (Boudry et al., 1993) or *Raphanus sativus* × *R. raphanistrum* (Panetsos and Baker, 1967). Likewise, the extent of gene introgression from an allopolyploid crop to one of its relatives depends on the genome on which the gene is located; for instance, introgression of a transgene from oilseed rape (AACC) into field mustard (AA) is easier when the transgene is located on the A rather than the C genome of the crop (Mikkelsen et al., 1996; Metz et al., 1997; Tomiuk et al., 2000). When hybrids are formed between less related species, the chance for gene introgression varies with the genomic structure of the hybrids. Although most hybrids contain each parental genome at the haploid level, some of them can also contain one of the parental genome at the diploid level due to the production of unreduced gametes (Eber et al., 1998), or both parental genomes at the diploid level (Kerlan et al., 1992). These different structures provide different opportunities for chromosomes to pair and can promote or preclude introgression. Intergenomic pairing is also modulated by genes superimposed on genome differentiation. While the presence of such pairing regulators has been suspected in different allopolyploid crops (Riley, 1963; Gauthier and McGinnis, 1968; Jauhar, 1993), genes promoting or preventing pairing have also been characterised in wild species (Riley, 1963; Eber et al., 1994) in which they are usually polymorphic (Dvorak, 1972; Taylor and Evans, 1977). Depending on the alleles, the wild plants are able to induce a high, intermediate, or low amount of chromosome pairing in crop-wild hybrids, which directly influences the frequency of introgression.

Moreover, the probability for introgression is not only a characteristic of entire genomes, but also a property of individual genes or chromosomal segments (Harrison, 1990). First, crossing-overs are not randomly distributed along the physical length of the chromosomes, but occur

more frequently in the distal rather than proximal regions of the chromosomes (Lukaszewski, 1995). It is not clear, however, if such a distribution is due to the chromosome segments themselves (recombination hot-spots) or to their position relative to the telomeres. Second, the colinearity between homoeologous or orthologous chromosomes is rarely conserved along their whole lengths, but is usually restricted to chromosome segments (Truco et al., 1996; Moore et al., 1997). The physical distribution of these genetically related regions should play a significant role in driving the amount and distribution of introgressed alleles, since recombination seems to occur preferentially between the most homoeologous regions (Delourme et al., 1998). Finally, the permeability of genomes to introgression depends on the number and genomic distribution of factors that contribute to reproductive isolation; basically, less introgression is expected for genes that are tightly associated with loci contributing to reduced fitness (Rieseberg et al., 1999), while chromosomal blocks can introgress at a higher rate when they contribute to advantageous gene combinations with positive fitness consequences (Rieseberg et al., 1996b; Burke et al., 1998). The architecture of reproductive barriers between species thus plays a tremendous role in controlling the extent and distribution of introgression events. If a large number of evenly distributed factors contribute to hybrid sterility and/or inviability, then the main part of the genome should be protected from introgression by selection (Rieseberg et al., 1996a; Rieseberg et al., 1999). Conversely, if few reproductive barriers isolate species, then most of the genome is expected to be permeable to introgression (Kim and Rieseberg, 1999).

Recombination and selection against recombinants can also interact in a complex manner. On the one hand, enhanced recombination provides greater opportunities to move genes across species because it promotes introgression and simultaneously reduces linkage drag; on the other hand, it can disrupt co-adapted gene complexes, thereby causing hybrid sterility and breakdown that interfere negatively with introgression (Li et al., 1997). In sunflower species, recurrent backcrossing to one or the other parent, which is likely when crop-wild hybrids occur at low frequency relative to their parents, proved to be an inefficient mechanism for breaking the parental linkage blocks compared to sib-mating (Rieseberg et al., 1996a). But populations resulting from sib-mating or selfing generally display higher levels of hybrid sterility and weakness than those derived by backcrossing (Rieseberg et al., 1996a). This balance is exemplified in Chèvre et al. (1997; 1998b) who observed

a restoration of fertility, but no transgene introgression, in advanced BC generations of hybrids between transgenic oilseed rape and wild radish. Tomiuk et al. (2000) provided additional support to the effect of fitness differences on chromosome transmission and introgression in interspecific hybrids.

Hybridization can have several outcomes other than introgression that can lead to the persistence of crop genes in natural communities. For example, allopolyploidy is thought to be the most frequent solution to the problem of interspecific hybrid sterility and segregation. The presence of either unreduced gametes or endomitosis after fertilization leads to production of fertile allopolyploid F1 hybrids containing each of the parental genomes at the diploid stage (Bretagnolle and Thompson, 1995; Ramsey and Schemske, 1998). The production of such amphidiploid plants has been reported among crop-wild spontaneous hybrids (David et al., 2000; Rieger et al., 2001). Similarly, Johnsongrass (*Sorghum halepense*), which is one of the world's ten worst weeds, is considered to be an allotetraploid hybrid between *S. bicolor* and wild *S. propinquum* (Paterson et al., 1995). Hybrid fertility and stability can also be restored without any change in the ploidy level (homoploid stabilization), through the sorting of chromosomal rearrangements in later-generation hybrids (Rieseberg, 1997). This process can lead to the formation of a new stable and fertile population that is homozygous for a novel combination of rearrangements. Detailed reviews have recently addressed the mechanisms controlling polyploidization (Bretagnolle and Thompson, 1995; Ramsey and Schemske, 1998) and hybrid speciation (Rieseberg, 1997). They show that the opportunity for gene spread through hybrid speciation depends on the stability of the genomes, the pattern of mating between the hybrids and their parents, the fitness of the plants, and the availability of a new ecological niche that can be colonized by the hybrids.

Fitness of wild-crop hybrids

Most workers who attempted to determine the extent to which crop transgenes may persist in natural populations have concentrated on measuring F1 hybrid fitness. Fitness may be defined as the relative ability of an individual to survive and successfully reproduce in a given environment, with the most fit leaving the greatest number of offspring.

Fitness is a function of a wide range of components throughout life: seed dormancy, germinability, vegetative growth, viability and male/female fertility.

Identifying the most critical components is not straightforward and depends on the ecology of crop relatives. For instance, some models predict that seed bank dynamics and seedling establishment under competition are the strongest determinants of the persistence of weedy ephemeral populations that occupy disturbed areas (Linder and Schmitt, 1994; 1995). By contrast, most authors have only examined the vegetative and reproductive productivity of hybrids (Tab. 2), because these parameters seem more directly related to the number of offspring one individual can potentially leave. Integrating results obtained on different lifetime components is difficult; it is not clear, for instance, if the lifetime fitness of *Brassica napus* × *B. rapa* hybrids is influenced more by their competitive advantage during the seed-to-seedling stage of their life (Linder and Schmitt, 1995) or by their reduced pollen fertility (Hauser et al., 1998a). Trade-offs between different components of fitness (investment in survival vs. reproduction, male vs. female allocation) raise additional problems and may result in spuriously confounded effects (e.g. Hauser et al., 1998a).

Notwithstanding the difficulty of identifying and combining critical components of fitness, many studies that have examined fitness-related traits in wild-crop hybrids showed that early crop-weed hybrids are not uniformly less fit than their parental forms (Tab. 2). Wild-crop hybrids sometimes demonstrate enhanced vegetative vigor that contributes to their total fitness, since competitiveness is related to plant size (Langevin et al., 1990; Hauser et al., 1998a). These results challenge the classical view that wild-crop hybrids should be less fit than their wild parents due to the burden that crop traits would introduce into wild plants (De Wet and Harlan, 1975; Small, 1984; Ellstrand and Hoffman, 1990). Current understanding of the genetic basis of domestication traits suggest that few genomic regions are usually involved (White and Doebley, 1998; Poncet et al., 2000) and can thus be purged quite rapidly, with no long-term impact on crop-wild hybrid fitness.

Even when wild-crop hybrids are on average less fit than their parents, they are usually so variable that some of them often demonstrate equivalent fitness components to the parental forms (Eber et al., 1994; Baranger et al., 1995; Mikkelsen et al., 1996; Hauser et al., 1998ab). Crop-wild hybrids will almost certainly not be genetically uniform due to variation in wild (and perhaps crop) populations (Linder and Schmitt, 1994). As a portion of this variation is heritable (Hauser et al., 1998ab), different donor and recipient populations should be used to test ideally for the fitness of hybrids

Table 2. Relative performance of wild-crop hybrids as compared to wild controls.

Wild relatives × crop species	Experimental setting	Measured traits	Relative performance	References
<i>Oryza sativa</i> (red rice) × <i>O. sativa</i>	F1 greenhouse	biomass production	hybrids > controls	Langevin et al., 1990
<i>Raphanus sativus</i> × <i>R. sativus</i>	F1 agricultural field without competition	seed production	hybrids ≥ controls	Klinger and Ellstrand, 1994
<i>Brassica rapa</i> × <i>B. napus</i>	F1 greenhouse	seedling emergence and vigor, biomass production	hybrids = controls	Linder and Schmitt, 1995
<i>Sorghum halepense</i> × <i>S. bicolor</i>	F1 agricultural field	biomass production, seed production, pollen viability	hybrids = controls	Arriola and Ellstrand, 1997
<i>Brassica rapa</i> × <i>B. napus</i>	F1 agricultural field	seedling survival, seed production	hybrids intermediate between controls	Hauser et al., 1998a
<i>Raphanus raphanistrum</i> × <i>R. sativus</i>	F1 outdoor pots and artificial populations	pollen viability	hybrids < controls	Snow et al., 2001
<i>Raphanus raphanistrum</i> × <i>Brassica napus</i>	F1, BC1 agricultural field	pollen fertility, seed production	hybrids < controls	Barranger et al., 1995
<i>Helianthus annuus</i> × <i>H. annuus</i>	F1 outdoor pots and weedy fields	seed dormancy, seed production	hybrids < controls	Snow et al., 1998
<i>Cucurbita pepo</i> × <i>C. pepo</i>	F1 agricultural field	seedling survival seed production, lifetime fecundity	hybrids = controls hybrids < controls	Spencer and Snow, 2001
<i>Hirschfeldia incana</i> × <i>Brassica napus</i>	F1 agricultural field	seed viability, seedling emergence	hybrids < controls	Chadoeuf et al., 1998
<i>Raphanus raphanistrum</i> × <i>Brassica napus</i>	F1 agricultural field	seed viability, seedling emergence	hybrids < controls	Darmency et al., 1998
<i>Raphanus raphanistrum</i> × <i>Brassica napus</i>	F1, F2 agricultural field	seed production	hybrids << controls	Darmency et al., 1998
<i>Raphanus raphanistrum</i> × <i>Brassica napus</i>	BC6 agricultural field	seedling emergence, pollen fertility, seed production	hybrids = controls or hybrids << controls ¹	Guéritaine et al., 2002
<i>Brassica rapa</i> × <i>Brassica napus</i>	F2 and BC2 agricultural field	seedling survival, seed production, pollen viability	hybrids << controls	Hauser et al., 1998b

¹: Guéritaine et al. (2002) obtained different results depending on the cytoplasm of the hybrids and the presence of an additional chromosome carrying a transgene that confers tolerance to an herbicide. Susceptible hybrids with a wild radish cytoplasm displayed equivalent performance as controls while hybrids with an oilseed rape cytoplasm displayed a sharp decrease in fitness.

(Karieva et al., 1994; Hauser et al., 1998a; Spencer and Snow, 2001). Variation in fitness is also expected across the subsequent hybrid generations due to recombination and selection. Hauser et al. (1998ab) observed that the fitness of *B. napus* × *B. rapa* hybrids sharply decreased in the second generation of hybridisation, with F2 hybrids demonstrating the lowest survival and reproduction. Hybrid classes that are genotypically more similar to one of the parents generally display equivalent fitness components as compared to the parental form. This trend has been strikingly exemplified among (*B. rapa* × *B. napus*) × *B. rapa* hybrids (Mikkelsen et al., 1996); the highest pollen fertilities (> 90%) were observed among the hybrids that had a genomic structure close to *B. rapa* (2n = 20 chromosomes).

According to Linder et al. (1998), estimates of early generations of crop-wild hybrid fitness may be of little predictive value for the assessment of transgene establishment unless hybrids are completely sterile. The long-term effects of a partial fitness barrier, *i.e.* when some hybrids survive but suffer from reduced fitness, are not clear. For instance, natural hybridizations between *Helianthus annuus* and *H. petiolaris* have resulted in at least three hybrid species (Rieseberg, 1997) despite very low fertility in the early generations of experimental hybridization. It must also be acknowledged that most studies have been performed under agricultural conditions, where water and soil nutrients were not limiting and inter-specific competition was low. The reproductive and vegetative performance documented in these experiments could have been different if relative hybrid fitness was evaluated outside of a cultivated field (Snow et al., 1998). Finally, very few studies have directly addressed the fitness of transgenic wild-crop hybrids (Linder and Schmitt, 1994; 1995; Snow et al., 1999; Spencer and Snow, 2001; Guéritaine et al., 2002). The fate of wild-crop hybrids and the spread of a transgene in natural communities will also depend on the nature of the genetically engineered traits.

Fitness effects of transgene(s)

Evaluating the spread and persistence of a transgene in free-living populations requires an understanding of whether possession of the transgene imposes a cost (“fitness penalty”) on the plant in the absence of selection for this gene, whether it confers a selective advantage, or whether it is selectively neutral. Genes with different selective value are expected to pass through a species barrier at different rates even when the fitness of hybrids

is rather depressed (Harrison, 1990). Clearly, these questions cannot be addressed globally.

First, the costs and benefits of transgenes depend largely on the phenotypic traits they confer to the wild plants. Some traits (production of pharmaceutical chemicals, modification of seed oil composition) are unlikely to be beneficial to free-living relatives; by contrast, genetically engineered herbicide tolerance, disease resistance and abiotic stresses tolerance are likely to enhance the fitness of plants carrying the transgene (Stewart et al., 1997; Snow et al., 1998). Second, the balance between costs and benefits is greatly affected by the nature and permanence of selective pressures acting on the transgene. For instance, resistance to a specific herbicide should only be beneficial to wild plants when and where the specific herbicide is frequently used, but this trait may be useless in most other natural communities. As exposure to biotic and abiotic stresses occur more continuously, genetically engineered tolerances to pest and abiotic stresses should enhance wild plant fitness in a wider range of habitats. Finally, the persistence of a transgene in wild populations not only depends on the balance between the costs and benefits this transgene impose on the wild plants, but also on the strength and frequency of gene flow. Population genetics theory predicts that strong and recurrent gene flow is sufficient to establish genes in populations where they contribute to slight fitness disadvantages (Haldane, 1930). The frequency of crop genes can thus be expected to increase in the wild populations if crop-to-wild gene flow is strong enough, irrespective of whether hybrids show increased or decreased relative fitness (Gliddon, 1994).

A complete treatment of the costs and benefits of transgenes is beyond the scope of this review, but several points can be briefly discussed. First, very few studies have attempted to evaluate the cost and benefit of transgene in crop-wild hybrids (*e.g.* Snow et al., 2001; Spencer and Snow, 2001; Guéritaine et al., 2002). Snow et al. (1999) demonstrated that there was no apparent fitness difference between transgenic and non-transgenic advanced hybrids of *B. napus* and *B. rapa*. Linder and Schmitt (1995) found little difference in the performance of oil-modified *B. napus* × *B. rapa* seeds and seedlings as compared to non-modified controls. Some experiments have compared transgenic versus non-transgenic cultivated plants, but these studies cannot be used to predict the effects of transgenes on the invasiveness of weedy species. Although minimal cost must have been selected for in commercialized crops (Purrlington and Bergelson, 1997), transgenes may

have different effects in another genetic background (pleiotropy and/or epistasis) or environment (Snow et al., 1999). Second, increased fitness of transgenic crops can increase the likelihood of introgression over evolutionary time by making it easier for crop individuals to persist in natural communities. Stewart et al. (1997) observed that *Bt*-transgenic *B. napus* had a better overwinter survivorship when selection pressures are exerted by insects. Conversely, Crawley et al. (1993) found no evidence for enhanced weediness in transgenic *B. napus* expressing herbicide tolerance. Finally, costs of resistance are expected to be more evident in a stressful environment (Reboud and Till-Bottraud, 1991; Bergelson, 1994). It has been suggested that when resources are abundant enough, excess resources can be channelled into resistance expression without significant consequences on fitness.

CONCLUSION AND PERSPECTIVES

To date, most studies have taken a short-term view of how transgenes may disperse and establish, and very few studies have assessed the extent of crop-to-wild hybridization in normal agronomical conditions (Wilson and Manhart, 1993; Whitton et al., 1997; Chèvre et al., 2000; Wilkinson et al., 2000; Rieger et al., 2001). In light of both theoretical and empirical data, our review suggests that gene dispersion will be very difficult to preclude totally. The most intuitive predictors of transgene escape (e.g. breeding system, relative ploidy level of both crop and weeds) are not sufficient on their own to correctly assess the associated risk. Crop-to-weed gene flow appears to be highly idiosyncratic and may be considered as crop-specific, and to some extent, variety-specific, site-specific or even season-specific (Raybould and Gray, 1993). Early crop-wild hybrid fitness and the intrinsic costs and benefits of transgene are important parameters that must be taken into account when considering gene establishment (Fig. 1), but they are not sufficient to draw definite conclusions about the associated risks. Natural selection will act on transgenic organisms or hybrids as it does on all others. Selection after the release of the transgenic plant will tend to increase fitness, not decrease it, by reducing the cost directly or indirectly associated with the novel traits (Tiedje et al., 1989). An important prerequisite is thus to verify that some genotypes will survive and reproduce at each generation, and thereby provide many opportunities for natural selection to operate.

Studies dedicated to assessing transgene escape and establishment have provided a wealth of useful

information and challenged the previous paradigms, such as the assumptions that crop-to-wild hybridization will never occur or that crop-wild hybrids will be systematically unfit, sterile or not viable. The future directions for research should question the long-term establishment and effects of transgene on natural communities, especially with regard to:

- *The ecology and population dynamics of weeds and feral crops.* The size, demography, and structure of weed populations can be critical determinants of the spread of transgenes, but are still difficult to apprehend. A proper evaluation of crop seed dispersal, establishment and persistence in wild communities is also required to understand the role of escapees in crop-to-wild gene flow.

- *A more accurate understanding of the way the different factors shape the distribution of introgression and interact with one another.* This information is important to predict the risks for transgene escape into wild communities and to identify “safer” insertion sites for transgenes (i.e. where the chance for subsequent introgression is reduced).

- *The efficiency and consequences of the different strategies proposed to reduce crop-to-wild gene flow.* Different confinement strategies have been proposed, but only a few have been tested. For instance, Morris et al. (1994) have shown that narrow barren zones are inefficient, and that trap beds increase gene escape when the source and recipient populations are growing nearby (Manasse, 1992). Only wider zones of trap crop captured more escaping pollen. Further studies are now required to test the efficiency and consequences of complete self-fertilizing varieties for partially autogamous crops (e.g. oilseed rape) or male-sterile lines for species used for their vegetative products (e.g. poplar or cauliflower). Similarly, genetic strategies that aim to reduce seed loss at harvesting and dormancy or to cope with herbicide tolerant volunteers are useful to reduce the impact of temporal transgene dispersal and must thus be investigated.

- *The development of genetic studies at the population level* – population genetic theory has challenged the classical belief that wild-crop hybrids must be as fit or more fit than wild plants in order for the engineered crop genes to persist and establish in the wild (Ellstrand and Hoffman, 1990; Klinger and Ellstrand, 1994; Scott and Wilkinson, 1998). But as Gliddon (1994) rightly emphasized, “although population genetic theory allows certain approximate statements to be made concerning the fate of genes introgressed from transgenic crops, there has been insufficient work on more ecologically realistic

population structure to allow for reasonable inferences to be made concerning rates of spread of genes". Thus, more studies that integrate ecological and genetic information are needed to allow a more accurate prediction of the fate of introgressed genes. Moreover, if hybridization may be a stimulus for the evolution of invasiveness (Ellstrand and Schierenbelk, 2000), the different factors that are responsible for the shift between the different steps of invasiveness must be understood in order to predict the risks due to crop-to-wild hybrids. Ecological and evolutionary studies are also needed to predict the evolutionary response of free-living populations (crop relatives, pest) to the genetically modified organisms.

Finally, as already stressed by Ellstrand et al. (1999), the question of crop-to-wild gene flow should not be restricted only to the case of transgenic organisms. Considering the potentialities and extent of crop-to-wild gene flow is also relevant for genetic resource conservation. Until now, few studies have addressed this question (Bartsch et al., 1999; Jenczewski et al., 1999).

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