

# **Hybridisation and introgression between crops and wild relatives**

Current knowledge and research priorities  
in lieu of impending introductions of GM crops

COGEM research 2003-02

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in lieu of impending introductions of GM crops

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## Preamble

This report was assigned by COGEM (the Netherlands Commission on Genetic Modification), advisory body for VROM (Ministry of Housing, Spatial Planning, and the Environment). It is meant to uncover possible lacunas in scientific knowledge in the field of crop-wild relative hybridisation and consecutive introgression of crop genes into wild relatives, as well as its ecological and evolutionary consequences. The motivation for this enquiry comes from the impending release of GM crops; the assessment and the *a posteriori* monitoring of the possible effects of such releases should be based on the best available scientific knowledge. The report reviews the current data and identifies the main research priorities to be implemented for further progress in this field.

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## Executive summary

Gene flow from genetically modified (GM) crops to their wild relatives or to other, conventional crops has recently raised concerns among both scientists and the general public. It underlined that the potential negative effects of the use of genetically engineered crops could perhaps outweigh the potential agronomic benefits of their introduction. Examples of such negative effects – or ‘risks’ - concern: (i) Establishment of the crop as a new weed, both in natural and agricultural environments; (ii) Formation of “superweeds” by hybridisation of a (wild/weedy) species with the transgenic crop; (iii) harm to non-target species; (iv) disruptive effects on biotic communities; and (v) genetic erosion (loss of original diversity of wild relatives). This study presents an overview of the current literature on the frequency and consequences of gene flow from crops to their wild relatives. The goal is to assess how this may help us to adequately perform Risk Assessment (RA) and monitoring procedures for GM crops, and also to identify in what areas more research is needed. In the debate on GM plants, “risk” is seen as a function of the effect of an event and the likelihood of the event occurring. Thus, the total risk depends on the probability of gene flow, and - once taken place - its effect on the recipient taxon and its environment.

## Probability of gene flow

Potential contacts between crops and wild relatives have been possible since the start of agriculture, and are not at all limited to GM crops. The general conclusion of this review is that, although levels of gene flow between crops and wild relatives vary, in most cases gene flow between them has occurred and will continue to occur. Moreover, further introgression after initial hybrid formation was found in a number of cases (*a.o.*, Carrot, Sunflower, Strawberry, Turnip). The fitness of hybrids and subsequent (backcross) generations is being increasingly studied, because hybrid fitness, in turn, will determine the subsequent effects of gene flow. Quite a number of studies on fitness of hybrids, with and without transgenic traits involved, are documented in the recent literature. The results were variable: sometimes hybrids were at least as fit as, or even more so than the wild relative, in other cases the reverse was found.

Crop-to-crop gene flow can be expected in virtually every cultivated species that is grown to the reproductive phase. Therefore, prevention of gene flow through engineered barriers (such as male-sterility or constructs preventing continued reproduction) and physical barriers (such as geographic separation of growing sites) is recommended for all traits with unwanted non-target effects or for which protection is called for other reasons. The latter is relevant for co-existence of GM practice with organic culture where GMOs are not accepted. Up-scaling of experiments should provide new information and, so far, often showed the underestimation of pollen dispersal made in small-scale field trials.

## Effects on a recipient population and its community

The effects of hybridisation and introgression were studied in much less detail than the occurrences of gene flow. It became clear that there is little information for many species and traits, especially concerning the long-term effects. More refined methods to measure fitness effects, and actually assessing the extent to which transgenes enhance or reduce fitness in natural settings are needed.

In particular, the potential effects of the use of transgenes that presumably affect the recipient's fitness - such as apomixis genes or stress tolerance genes - have hardly been studied, if at all, let alone the subsequent consequences at the community level. Demographic monitoring could prove a very powerful tool in establishing the life cycle stages that are most relevant to population survival and growth. Firstly, this would enable us to evaluate the pertaining traits for their influence on critical stages during a taxon's life cycle and therefore, for the establishment, survival and spread of hybrids in the field. Secondly, it may also give insights in the selective pressures acting on transgenes during the process of introgression, and thus which genes are more (or less) likely to spread.

## Lacunae in our knowledge

Ultimately, introgression and persistence of (trans-)genes in wild populations depends on many aspects. It is evident that, in order to estimate a transgene's tendency to introgress and persist in wild relatives, all parts of the introgression chain need to be considered. We listed the studies in which crop-wild relative gene flow was documented up to the phase of introgression of genes into the wild species. Relative to the number of hybridisation events, in-depth data on the actual follow-up are as yet scanty. Much more information on, for instance, the effects of the transgene insertion site on the introgression process and the importance of fitness of the intermediate stages (backcrosses) is needed to reach more general insights. Very few studies have reached the stage in which the environmental and evolutionary effects of hybridisation and introgression are documented.

This report identifies a number of lacunae in our knowledge of introgression and its consequences, in particular in the following areas:

- Actual rates of outcrossing and introgression in less studied crops, including reliable methods to monitor outcrossing in the field;
- Fitness effects of relevant traits under natural conditions in relation to transgene persistence after escape;
- Fitness effects of hybridisation in general and whether specific parts of the genome are more likely to introgress into surviving hybrid offspring;
- Causes of weediness and invasiveness in general and for crops and their relatives in particular;
- Occurrence of swamping of wild type natural populations by hybridisation and crop volunteers;
- Integrating the above into a spatial model on landscape scale, including up-scaling to agricultural population sizes.

## 1. Introduction

Gene flow in its broadest sense is the dispersal of genes (by way of pollen grains, seeds, or vegetative reproduction units) from one population of a taxon to another. Implicit in the concept of gene flow is that (at some stage of the process) haploid genomes from the parental taxa indeed are combined in a zygote that develops into a new individual, so that the incoming genes are incorporated in the gene pool of the receiving population. The process starts with the arrival of pollen, seeds or other propagules in a focal population, and the fate of these incoming units determines whether gene flow actually occurs or not. Gene flow as such is a common and natural phenomenon, (accountable for genetic species coherence and thus also for defining the limits of the biological species as well as for the presence of hybrids between species, and it delivers the raw material for local selection and ultimately evolution to take place. Gene flow between crops and wild relatives has been an integral part of agricultural development from its onset in the Neolithicum.

One should realise that crop plants establishing feral populations - and eventually developing invasive characteristics - can also be seen as a form of gene flow, and indeed from many crops feral population complexes are known to have established, *Brassica rapa* being a well known example. This study, however, is restricted to the process and consequences of incorporation of genes into the wild type gene pool through hybridisation(s) and introgression. In this literature study we present an overview of the current state of knowledge with respect to gene flow that may exist, or has occurred already, between crop plants and wild taxa that are closely enough related to produce viable hybrids after cross-pollination.

Recently, potential gene flow from genetically modified (GM) crops to their wild and weedy relatives and/or conventional crops attracted a lot of attention. In genetically modified (i.e., transgenic) plants a short fragment of DNA is inserted and integrated into the genome of the plant. The DNA can be derived from (unrelated) other living sources (plant or non-plant), and is usually engineered by combining a coding gene with appropriate regulatory and reporting sequences. Being able to recombine genomic material from unrelated sources clearly opens possibilities in crop breeding beyond those of traditional breeding, and genetically engineered crops are advocated for a number of benefits. Crops could be modified in a way to increase yields or to extend the area in which they can be grown (direct economic benefits); alternatively, benefits may come from reductions in the use of insecticide or fertiliser (agronomic and/or environmental benefits). At the same time there are many concerns about potential harmful effects of their introduction, and unintended outcomes of the use of genetically modified organisms (GMOs) appear to be possible (Tiedje *et al.* 1989). Examples of the risks mentioned in the context of GMO use are: (i) Development of new weeds resulting from the crop establishing feral populations; (ii) Formation of super-weeds by hybridisation of a (wild/weedy) species with the transgenic crop; (iii) Harm to non-target species (for example: beneficial insects (such as pollinators, parasites of plague species, soil organisms) could be negatively affected by genes inserted in a crop plant); (iv) Disruptive effects on biotic communities (altering community composition by, for example, diminishing seed predation of transgenic

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plants or hybrids through introduction of genes coding for insecticidal toxins); and (v) Genetic erosion (loss of original diversity of wild relatives).

To avoid unintended and undesired effects of any introduction of a GM crop, a thorough regulatory system has been implemented, according to which in-depth Risk Assessment (RA) studies are to be performed before market consent for a transgenic crop variety can be applied for. RA's need to be performed on a case-by-case basis, given the notion that each engineered trait may have its specific effects depending on the crop used. On the basis of this regulatory axiom, generalization of risk assessments is difficult, if not formally impossible. Nevertheless, it is clear that there are also general ecological and population genetic rules that govern gene flow events and their consequences. Some of these rules apply to a given combination of crop and wild relative rather independent of the introduced gene. For instance, it is obvious that hybrid sterility is a strong barrier to further gene flow and introgression, in which case attention can be focussed at the incidence and fate of first-generation hybrids only.

Because the term Risk is often misunderstood, we like to very briefly restate the concept of Risk and Risk Assessment as it is implemented in this field. In the debate on GM plants, there is much confusion as to how to use the term "risk". Many biosafety research studies tended to regard "risk" as equivalent to "exposure" or assume that "gene flow" equals "hazard". If this were the case, even evolution would be classified as a harmful process, since hybridisation, introgression and gene flow is essential to speciation, especially in plants. The more neutral definition of risk is that risk is a function of the effect of an event and the likelihood of the event:

$$\text{Risk} = f(\text{effect, likelihood})$$

In this formula, effect is synonymous to hazard, which has a negative connotation. However, not all effects are negative by definition. The outcome of the risk assessment must be that all negative effects connected to the commercial exploitation of the GM plants are identified and quantified so that these can be balanced against positive effects. The factor likelihood can be equated to the probability of gene flow, so that the total risk is the outcome of the gene flow that occurs and its effect on the recipient taxon and/or the community it belongs to.

It is obvious that regional differences in ecology and agricultural practice (climate, substrate, co-occurring biota, crop rotation) will cause variation in the frequency of events as well as their consequences. For instance, regional differences in the presence/absence of crop relatives will obviously affect gene flow with the crop. Such considerations lead to the conclusion that a biogeographical assessment of wild relatives' distribution patterns (and inherent variation in reproductive traits), as well as the agro biodiversity pattern should be part of the baseline data set available to risk assessment and monitoring procedures. This would include aspects as the regional spectrum of genetic variation in land races, the composition of plant communities in the immediate surroundings of the areas in which GMOs are grown, and the entomofauna (such as pollinators) associated with the species.

It is relevant to realize that after a market introduction, the growing area of a crop is much bigger than in an experimental phase. Consequently, the effects of up-scaling should be recognized, and differences at the international and biogeographical level have to be taken into account. An introduced, transgenic crop could become a pest in some areas/countries, whereas in the environment where the crop was produced initially it never developed into a problem. Thus, international co-ordination of risk assessment and regulation of biotechnology will be a very important issue in the research on GMO-use.

One of the target areas for in-depth analysis is introgression, being defined as the assimilation of genes of one population or species into the gene pool of another population or species. Concerns about the introgression of transgenes into wild relatives raised questions regarding three aspects: the rates of hybridisation and introgression between cultivars and their wild relatives, the fitness and the ecological behaviour, for instance the invasiveness, of hybrid and backcross generations, and finally the fate of taxa that might suffer from genetic erosion or undergo genetic swamping, due to continued transfer of genes from a crop.

In this study, we give an overview of research on these subjects of hybridisation and the subsequent introgression (if any) of (genetically modified and traditional) crops and wild relatives, and its impact on the environment. The aim is also to identify possible lacunas in our knowledge of the likelihood of gene flow and the effect of hybridisation and introgression. Is the current state of the art sufficient for assisting adequate risk assessment studies and (EU and nationally prescribed) monitoring of effects after the introductions of current and future GM crop races?

This report forms part of a series assigned by COGEM, other studies investigated current knowledge (and gaps) in the broad area of potential effects on soil biota and multitrophic above-ground relations, see Bruinsma *et al.* (2002) and Knols and Dicke (2003), respectively.



## 2. Introgression from (GM-) crop into wild relatives

Gene flow is a common phenomenon in nature, and it is not surprising that it occurs between crops and their wild relatives, among some of which are indeed their ancestors. Presumably the most pertinent question to answer is therefore: are the hybrids vital enough to reach the reproductive phase and produce offspring? Hybrid fitness is the essential factor that determines the effectiveness of the initial gene flow. If subsequent generations of hybrids can be established, questions concerning the long-term effects of the introgression process need to be answered.

In this chapter, we will review the following aspects:

- what do we know about the rate of gene flow between crops (of any kind) and their wild and/or weedy relatives?
- to what extent are viable F1 hybrids between crops and wild specimens formed, and what is known so far about their fitness?
- is there subsequent introgression, in other words, to what extent do follow-up generations of the initial hybrids occur, and do they lead to actual incorporation of crop genes in the acceptor wild genomes?
- what is known about the ecological and evolutionary effects of the introgression, once taken place?

Table 1 gives a concise overview of the above four aspects, ordered per crop, *viz.*, their respective wild relative species, to give insight into the current knowledge on different crops and in the amount of information available per topic. The table makes clear that the farther into the introgression process, the fewer studies and data are available.

### 2.1 Occurrence of Crop to Wild relative Gene flow

Ellstrand *et al.* (1999) showed that 12 of the 13 most important food crops of the world hybridise with wild relatives in some part of their agricultural distribution (see Table 1). Also other reviews suggest that gene flow from crop to wild relatives is probably the rule rather than the exception. Raybould & Gray (1993) reviewed 31 domesticated plant species in the United Kingdom and found that about one third spontaneously hybridises with one or more elements of the local flora. In The Netherlands, the result was about one quarter of 42 reviewed species (de Vries *et al.* 1992). Ellstrand *et al.* (1999) explain these results in their evolutionary context, domesticated plants representing lineages that diverged from their progenitors no more than a few thousand years ago. Clearly, reproductive isolation cannot be assumed to be the general rule, and from Table 1 it must be concluded that gene flow from traditional crops as well as from some GM crops to wild/weedy relatives is taking place on a broad scale.

The key condition for gene flow and introgression is, of course, the presence of wild or weedy relatives in the vicinity of the crop. Generally, pollen flow from a specific source follows a leptokurtic distribution, which means that the great majority occurs

over a short distances; long distance dispersal is rare, but also quite variable and thus rather unpredictable. For instance, Klinger *et al.* (1992) found that distance alone will not act to completely isolate populations of radish (*Raphanus sativus*) and prevent gene flow and hybridisation between crop and wild relatives. This result was also found in a study of sunflower (*Helianthus annuus*) by Arias & Rieseberg (1994), who found gene flow occurring up to 1000 m from the crop. Moreover, in a re-analysis of the data on radish, Klinger (2002) showed that even for this obligatory outcrossing species it is difficult to predict the actual rate of gene flow in the field; variation between experiments in the rate of pollination was high, particularly so at larger distances from the source. In addition, Reboud (2003) showed that a gap between crops is inefficient in reducing pollination by insects: an unplanted distance of 3-4 m had no more effect than one of 1m.

Spatial reproductive isolation by ecological barriers like woods, hedges or a surrounding planting of a different crop is often used to prevent crop pollen from dispersing to other plants in the surrounding vegetation. Dense stands of shrubs, herb covers and tree-sized vegetation with full foliage act as traps for airborne particles, including pollen (Treu & Emberlin 2000). The nature of the plant canopy, surrounding vegetation and topography can affect wind velocity and airflow, thereby influencing pollen movement from the pollen source to receptor plants (Eastham & Sweet 2002). Jones & Brooks (1952) conducted experiments with a tree barrier and concluded that this was effective for plants growing near the barrier, but much less so at greater distances. Barrier crops (a border of non-GM plants of the same crop surrounding the GM variety) can act as an 'absorber' of GM pollen and Morris *et al.* (1994) suggest that the most effective strategy would be to plant the area surrounding the crop with a trap crop that could be destroyed before seed set. Obviously, this is a rather costly practice. Reproductive isolation by means of differences in flowering time can also act as a barrier that prevents hybridisation; in practice, however, the flowering periods of crops and the wild relatives are often overlapping.

Other reproductive barriers such as sexual (in-)compatibilities may play an important role in the frequency of hybridisation. Post- and pre-zygotic barriers have been found in a number of species. Lefol *et al.* (1996) showed that, for example, between genetically modified oil seed rape (*Brassica napus*) and hoary mustard (*Hirschfeldia incana*) two types of genetic barriers exist, both pre-zygotic. The first barrier is reduced pollen germination and growth on the papilla of the recipient species. The second, later barrier is the weak attraction of foreign pollen tubes to the micropyles of ovules, hampering successful fertilisation and seed formation. An example of a post-zygotic barrier was found between oilseed rape and wild radish (*Raphanus raphanistrum*). Presumably, a functional incompatibility between oilseed rape cytoplasm and the wild radish nuclear genes resulted in a low seedling emergence, low survival, limited development and chlorophyll bleaching; this only occurred in crosses in which oilseed rape was used as the mother plant, and thus as donor of the uniparentally inherited chloroplasts (Guéritaine *et al.* 2002).

Since F<sub>1</sub> hybrids are assumed to be rare in some crop/wild combinations and they can be sterile or have a very low fecundity (e.g. Snow *et al.* 1998), only few of the hybrid

populations will be able to persist (Raybould & Gray 1993), unless they are able to reproduce vegetatively or remain in contact with parental taxa. The latter would lead to backcross generations, and actual introgression of genomic crop elements in the wild relative. However, only fully sterile  $F_1$  hybrids would completely prevent the further transfer of genes into wild/weedy relatives.

### ***Conclusions***

The general conclusion from the above summary and the data in Table 1 is that levels of gene flow vary with a range of factors: (i) it depends on the cross-compatibility between the crop and its wild conspecific, or related species from the same or a different genus; (ii) the cross-compatibility both within and between species may vary depending on the genotypes involved, (iii) it may include pre-zygotic as well as post-zygotic barriers; (iv) temporal barriers (different flowering periods) and spatial barriers, like barrier crops or hedgerows, or sheer distance, may play a role; and (v) environmental conditions, such as prevailing winds, availability of pollinators, growth conditions during seed set, etc. all can affect gene flow.

Even though chances may vary, in many crop-wild relative complexes sooner or later gene flow will occur, and may lead to further introgression. Therefore, it is imperative to know the consequences of introgression, i.e., the fitness of  $F_1$ -hybrids and subsequent generations. Fitness of hybrids, in turn, will determine the chances for genes to establish in the recipient taxon/population, and, consecutively, for a continued spread of the pertaining traits in the population. If so, chances of (local) extinction, due to genetic swamping, for instance, need also be taken into account.

## **2.2 Fitness of hybrids**

Hybridisation is only the first step in the process of introgression. If the initial hybrids are sterile or not viable further spread is automatically blocked. Only in case hybrids reach the reproductive phase and produce offspring the process of introgression proceeds. Thus, it is highly relevant not only to establish the potential and occurrence of hybridisation between taxa, but also to assess the fitness of the hybrid generation.

It is often assumed that crop genes, particularly the ones associated with domestication, (and perhaps also transgenes) will be disadvantageous to wild populations or will have certain detrimental/fatal fitness effects on hybrid populations (Small 1984), and that they therefore will not be able to spread. However, genes involved in domestication often show recessive domestication alleles, so that in a cross between a crop plant and a wild relative the domesticated allele will be overruled at the heterozygous state, and thus the hybrid will express the wild type phenotype. If so, first generation hybrids will be similar to the wild parent, which may increase their chances of survival in the field (Papa & Gepts, 2004 in press). In later generation hybrids, crop traits incorporated in the wild relatives may come to expression, resulting in reduced fitness outside agricultural fields (Small 1984).

An example of such a maladaptation was shown by Hauser (2002); he found that hybrids between cultivated carrot (*Daucus carota* ssp. *sativa*) and wild carrot (*Daucus carota* ssp. *carota*) inherited the sensitivity to frost from the cultivar parent and therefore had a much lower survival than the wild carrots and a slightly better survival than the cultivar. This indicated that frost is expected to decrease the survival of hybrids. A similar situation was found in oilseed rape (*Brassica napus*), weedy Turnip (*Brassica rapa*) and their hybrids: Landbo & Jørgensen (1997) showed that hybrid seeds (from reciprocal crosses) resembled the crop parent, *Brassica napus*, in expressing no dormancy. Thus, hybrids are not able to spread the risk of germination in unfavourable environments by building up a seed bank in the soil, suggesting low hybrid fitness under (unpredictable) field conditions. Another seed character that can affect hybrid survival is seed size, as shown by Alexander *et al.* (2001). Hybrid seeds, produced in crosses between crop and wild sunflowers (both *Helianthus annuus*), were twice the size of wild seeds and were eaten significantly more than the wild seeds. Therefore, these authors concluded that differential predation reduced hybrid fitness. Note, however, that seed size is usually regarded as a positive indicator of fitness, for instance because more food reserves lead to enhanced seedling survival. Apparently, the trade-off between advantageous aspects of seed size and predation should be carefully evaluated to assess the net fitness effects in the field. Snow *et al.* (1998) also investigated the fitness of F<sub>1</sub> hybrids in sunflower. They performed seed burial experiments to assess seed dormancy and also quantified lifetime fecundity and flowering phenology of hybrid and wild progeny. The results suggested that F<sub>1</sub> wild-crop hybrids had lower fitness on average than wild genotypes, i.e., exhibited less dormancy and a lower seed production. However, there were notable exceptions at specific field sites, such as an equivalent level of dormancy or resistance to a rust fungus affecting the wild population. Two examples of neutral fitness effects were demonstrated by Burke and Rieseberg (2003) for wild mold resistance (also in *H. annuus*), and by Bartsch *et al.* (2003) for a viral resistance gene in *Beta*, respectively.

In contrast with the previous findings, in many taxa interspecific hybrids were found that are as fit as or even more fit than their parents (reviewed by Arnold 1997). For example, Hauser *et al.* (1998b) found that hybrids between weedy *Brassica rapa* and cultivated *Brassica napus* were intermediate in fitness between their parents and significantly more fit than weedy *B. rapa* as determined by seed characteristics and survival in the field. Furthermore, interspecific hybrids between the crop sweet sorghum (*Sorghum bicolor*), and the noxious weed Johnsongrass (*Sorghum halepense*) showed no significant fitness increase or decrease in comparison with the non-hybrid *S. halepense* under agricultural conditions (Arriola & Ellstrand 1997). On the other hand, the relative fitness of hybrids between crop and weedy Radish (*Raphanus sativus*), exceeded that of their wild siblings in an experiment conducted by Klinger & Ellstrand (1994). They measured germination success, time of first flowering, fruit production and seed production of weedy radishes and their sibling weed-crop hybrids; hybrids showed significantly greater fruit and seed production. As can be seen from the above examples, hybrids between crop and wild species can be sufficiently fit to survive and reproduce, which could lead to the spread of crop genes. Generally, the transfer of (trans)genes from a crop to wild or weedy relatives is more likely to

succeed if the trait is neutral or beneficial to the hybrid population. Dietz-Pfeilstetter & Kirchner (1998) manually outcrossed a transgenic BNYVV (Beet Necrotic Yellow Vein Virus, causing the disease rhizomania) resistance based on the virus's coat protein gene to two accessions of the wild beet (*Beta vulgaris* ssp. *maritima*) and found an increased level of virus resistance in the F<sub>1</sub> hybrids from one of the wild beet accessions. F<sub>1</sub> hybrids from the other wild accession did not show enhanced virus resistance, but plants of this accession already had a high level of natural resistance to rhizomania. The increased virus resistance found in this experiment could lead an increased fitness of the hybrids in the field. In sunflowers, Snow *et al.* (2003) recently found evidence for increased fitness of F<sub>1</sub>-hybrids of Bt transgenic crop race and wild type material. In pumpkin, *Cucurbita pepo*, different fitness components of wild plants and wild-crop hybrids were compared. The crop parent contained a transgenic resistance to two viruses. The results suggested that the F<sub>1</sub> generation was vigorous enough to contribute to the gene pool of subsequent generations (Spencer & Snow 2001), but the hybrids were no more fit than the wild plants under the tested conditions. Whether fitness would be enhanced under conditions of virus pressure in the field was not established. Another study on trait-related fitness of hybrids was done by Oard *et al.* (2000) on transgenic rice. Here, the transfer of the BAR gene, which confers tolerance to glufosinate herbicide, from transgenic rice (*Oryza sativa*) to the noxious weed red rice (*Oryza sativa* forma *spontanea*) by hybridisation did not increase or decrease fitness values for traits such as dormancy or seed production, thus the reproductive success of the weedy hybrids was not expected to be influenced by the transgene. Another example of neutral fitness effects was demonstrated by Burke and Rieseberg (2003) for transgenic white mold resistance in *H. annuus*.

## Conclusions

Quite some studies on fitness of hybrids, with and without transgenic traits involved, have been performed, as also summarized in the reviews by Ellstrand *et al.* (1999) and Eastham & Sweet (2002). There is not a single conclusion from these studies. Effects vary from apparently absent to fitness reduction or enhancement. Effects of the few GM traits so far studied depend on taxon and circumstances. Herbicide resistance did not seem to influence fitness (positively or negatively) in non-agricultural settings, whereas Bt transgenes caused an increase in fitness related characters of seed survival. In general, it is clear (Table 1) that for many species data are still scarce. Aspects that particularly need more study are (i) assessment of the extent to which genes, such as those conferring resistance to biotic as well as abiotic stresses, affect fitness in natural settings, and (ii) extension of the methods for the assessment of fitness. The latter particularly by not merely studying fitness-related traits (such as germination rate, survival, fecundity) under controlled conditions, but also in relevant field situations. Furthermore, demographic monitoring in the field could establish the life cycle stages most relevant to population survival and growth, so that the fecundity traits could be put in the proper perspective, weighing their role in the life cycle on the basis of their relative influence on population growth. This approach would lead to a better insight in the traits' future establishment, survival and spread in the field (Oostermeijer, 2000; Luijten *et al.* 2002). The latter would hold even more if the demographic models could



be integrated with data from population and quantitative genetics (van Tienderen, 2004 in press).

### 2.3 Introgression

Introgression is the persistent incorporation and integration of genes from one population or species into the gene pool of another one. After initial formation of fit F<sub>1</sub> hybrids, the following stage of the process would be the incorporation into the gene pool of the recipient population of the “alien” genes. This aspect is particularly problematic to document since the relevant population-genetic process may take many generations to become effective.

A pre-condition for introgression from crop to wild is the occurrence of hybrids sufficiently fit to produce progeny in the field, as described in the previous chapter. In order to have a persistent effect on the genetic wild populations, first generation hybrids should be able to backcross with wild parental plants and produce fertile progeny. Persistence of cultivar alleles in wild populations of *Helianthus annuus* in generations following hybridisation was studied by Whitton *et al.* (1997). Two cultivar-specific RAPD markers were used to survey progeny of a naturally occurring population of wild *H. annuus* over five generations following a single generation of hybridisation with the crop. It is concluded that a long-term establishment of cultivar alleles in wild populations followed this hybridisation event.

Westman *et al.* (2004 in press.) argue that the chance of persistence of introgressed characters is greatest when plants are perennial, propagated by both seed and vegetative clones, and produce edible fruits dispersed by animals. Their research on strawberries, *Fragaria x ananassa*, and wild *Fragaria virginiana* by AFLP marker analyses indicated a potentially long-term persistence of introgressed markers in wild populations in the surroundings of cultivated fields.

AFLPs were also used in research concerning the introgression and persistence of cultivar and wild plant markers of carrots (*Daucus carota* ssp. *sativa* and *Daucus carota* ssp. *carota*, respectively) (Hauser *et al.* 2004 in press). A few of the wild plants close to the carrot fields were somewhat similar to the white-rooted bolters in the field and this would be expected if the bolters mated with the nearby wild plants. From the preliminary results of this ongoing study, the authors concluded that hybrids can be found in wild populations close to the crop and that they sometimes will be able to establish and persist in natural habitats. In addition, Hauser *et al.* (2004 in press.) showed that the hybridisation routes between cultivated and wild carrots via seed producing areas can be as important or even more important than gene flow directly from flowering plants in the cultivation areas.

In this regard, beet (*Beta vulgaris* ssp. *vulgaris*) is a particularly interesting case in that gene flow from wild to crop in the seed multiplication areas in southern Europe can subsequently confer crop to wild gene flow in the cultivation areas in northern Europe. In northern Europe, the biennial beet is usually harvested before the onset of

flowering, although bolters sometimes are present at harvest. Hybridisation in the seed production areas between sugar beet and annual/weedy forms of wild sea beet (*Beta vulgaris* ssp. *maritima*) can lead to contamination of seed lots for cultivation in northern Europe. Through this route, annual weed beets have established in beet cultivation areas and these weed beets may further facilitate gene flow from the crop to wild sea beet where beet cultivation occurs close to the coastal habitat of sea beet in northern Europe (Boudry *et al.* 1993). The creation of weed beets appears to be a relatively simple process, since the annual life history required for persistence in beet cultivation fields is based on a single dominant bolting allele B. This bolting allele can be picked up by cultivated beets through fertilization by pollen from annual beets in seed production areas (Boudry *et al.* 1993). Chances of transgene escape in beet could be lowered, but not excluded, by manipulation of the insertion site of the transgene, by inserting the transgene into tetraploid pollinator breeding lines (Desplanque *et al.* 2002). As far as has been ascertained up till now, exchange rates between weed beet and coastal sea beet were below detection level in French cultivation areas (Desplanque *et al.* 1999; Cuguen *et al.* 2004 in press). However, Bartsch *et al.* (1999) documented exchange between beet and sea beet in seed production areas in Italy (see further below under ecological/evolutionary effects). Also in the Ukraine, as Slyvchenko and Bartsch (2004 in press) showed using quantitative isozyme markers, introgression of cultivar alleles into the gene pool of the local sea beet populations has occurred. This result supported ‘*the contention that gene flow from the crop to the wild species can be substantial when both are grown in proximity*’ as is also noted by Hauser *et al.* (2004 in press.)

In the case of poor compatibility between crop and wild species, fertility can be restored in hybrid offspring when the hybrids backcross to the wild relative, as was shown for hybrids between oilseed rape and wild radish (Guéritaine *et al.* 2002). The descendants from such backcrosses can produce fertile pollen and consequently (trans)genes can move towards the wild relative and stable introgression of (trans)genes into wild populations can be achieved. On the other hand, Hauser *et al.* (1998a) found that backcrosses and F<sub>2</sub> hybrids between weedy *Brassica rapa* and oilseed rape (*Brassica napus*) had a reduced fitness relative to their parents for most of the fitness components measured, but some of the hybrids were as fit as their parents. Therefore, the authors suggested that, in spite of the low fitness of second-generation hybrids, introgression of transgenes would not be completely hindered.

As already indicated for beet, the chances of transfer of transgenes could be influenced by the integration site of the construct on the genome, as was implied by Metz *et al.* (1997). Hybridisation between two transgenic *Brassica napus* lines and non-transgenic *Brassica rapa* was studied and large differences were found in the transmission frequency of the transgene between the offspring of the two transgenic lines during backcrossing. *B. napus* is a so-called amphidiploid, which is supposedly derived from a species cross between *B. rapa* having the A genome of 2n=20 and *Brassica oleracea* having the C genome of 2n=18. This led to a tetraploid species hybrid containing both the A and C genomes from the respective ancestral species, leading to 2n chromosome number of 38. The genetic relationship between these Brassica species and three

additional ones is commonly known as the “U-triangle”, named after its creator. Thus, Metz *et al.* (1997) suggested that a construct situated on the C-genome (derived from *B. oleracea*) of *B. napus* might have limited the transfer to *B. rapa* (having the A genome). On the other hand, related species containing (parts of) the C-genome (e.g., *B. oleracea*) would then have an increased chance of introgression of the transgene. This view was challenged by Tomiuk *et al.* (2000), using a simple population–genetic model: results of Metz *et al.* (1997) could also be explained by selection against transgenic A-chromosomes during backcrossing; therefore, it would be necessary to first establish on which genome the transgene was integrated in the *B. napus* lines used. In the end, the safety of using specific integration sites would also depend on the amount of homologous and homeologous (i.e., between A and C genomes) recombination events.

In relation to the relevance of the position of the transgene on the parental genome, interesting observations came from studies of hybridisation between wild species from the sunflower genus *Helianthus*. Rieseberg *et al.* (1996) experimentally re-synthesised a known ancient hybrid between *H. annuus* and *H. petiolaris*, *H. anomalus*. The three different hybrid lineages created had a similar genomic composition as the hybrid species *H. anomalus*, as evidenced by a comparison of linkage maps made using molecular markers. Apparently only particular combinations of the parental genomes led to viable hybrid offspring. This would mean that chances for a transgene to end up in introgressed wild populations will depend on the insertion site and this would have to be taken into account when assessing safety. On the other hand, it is doubtful whether this would be a feasible approach to containment of transgenes in crops.

In another study on fitness of backcrosses of *Brassica napus* and *Brassica rapa*, Snow *et al.* (1999) looked at possible costs associated with the transfer of transgenic resistance to glufosinate. No significant differences in survival or number of seeds per plant were found between transgenic and non-transgenic plants and therefore it was concluded that the costs of the transgene integrated in the plants are negligible. They found also that pollen fertility and seed production of BC<sub>3</sub> plants were as great as those of the *Brassica rapa* parent, in contrast to the research of Hauser *et al.* (1998a) mentioned above.

In *Helianthus annuus*, integration of the Bt transgene also was not associated with any fitness costs (or benefits) in BC<sub>1</sub> plants as is shown by Pilson *et al.* (2004 in press.). However, in field experiments the transgenic backcross plants had a fecundity advantage due to protection from lepidopteran herbivores. It is expected that the transgene will spread quickly, because subsequent generations of Bt wild plants will probably be able to produce more seeds per plant than non-transgenic individuals, and the introgressed transgene increases fitness in the field. In this respect, knowledge of the demography of the pertaining species is important, since the effect of seed production on population survival and growth may be a crucial factor.

Also fitness-related traits like seed dormancy can be influenced by backcrossing. Especially when the hybrids backcross to their wild parent, the resulting progeny can adopt certain traits from the wild relative, among which seed dormancy, and this can

result in plants that are more adapted to the variable environmental conditions of wild populations, as for instance has been demonstrated in *Brassica* by Landbo & Jørgensen (1997). Such results indicate that (trans)gene escape in time, through the persistence of dormant seeds in the soil (Linder & Schmitt 1994), could happen by backcrossing of the hybrids with their weedy parent.

In addition, Linder & Schmitt (1995) assessed the chance that seed-oil-modification transgenes will increase the persistence of feral *Brassica napus* canola and hybrids of *B. napus* and wild *Brassica rapa*. They found different results for the two oil-modification transgenes used in the experiments and therefore suggested that even transgenes with similar functions should be considered on a case-by-case basis. Furthermore, the high-laurate hybrids may experience performance advantages: they emerged more rapidly and had greater biomass at two weeks than their hybrid controls; they may perform as well as their wild parent and therefore they would be able to persist outside the cultivated field.

### ***Conclusions***

It has been shown that introgression and persistence of (trans)genes in wild populations depend on numerous aspects. It is clear that, in order to estimate whether a transgene is capable of introgressing and persisting in wild relatives, all steps in the introgression process should be considered (Landbo & Jørgensen 1997). Table 1 shows in which crop-wild relative complexes studies have reached the phase of introgression. It is obvious that, given the number of hybridisation events, data on subsequent introgression is as yet scarce. More data on the effects of the transgene insertion site on the introgression process and the fitness of the intermediate stages (backcrosses) is needed.

## **2.4 Ecological & evolutionary effects of introgression**

Within the regulatory framework of Risk Assessment, any gene flow and introgression is the more of interest if there are consecutive undesirable effects on non-target organisms. The latter effects could range from herbivores to hyper-parasites, and, of course, can be the close relatives of the crop itself. Only effects on the non-target organisms formed by the interfertile wild relatives will be dealt with here. Among the unwanted effects are, as pointed out before, invigoration of weediness in already existing species, the *de novo* development of weedy traits in so far harmless taxa, changes in the ecological relationships within a community, and the loss of genetic identity in taxa that undergo genetic erosion or genetic swamping. As a sequel to the last chapters, we here will report to what extent this sort of evolutionary and ecological consequences of introgression have been studied.

As an example of enhanced weediness, the evolution of this character in one of the world's worst weeds, *Sorghum halepense*, is assumed to be a result of introgression from the crop *Sorghum bicolor* (reviewed by Ellstrand *et al.* 1999). Transgenes may enhance these chances, depending on the specific traits encoded.

Persistence of and consequent competition by crop-wild hybrids could potentially cause the loss of genetic variants unique to the wild species. Genetic markers can be used to examine loss of genetic variation after hybridisation, which is suggested by Westman *et al.* (2004 in press). They identified markers characteristic of wild and cultivated strawberry species, including markers for cultivars not being cultivated anymore, and this enabled them to assess the persistence of older cultivar markers in current hybrid populations. The results suggested that older cultivar markers persisted in populations in the surroundings of cultivated fields (the traditional cultivars were no longer grown there). They suggest an evaluation of the relative frequencies of the markers in wild *Fragaria virginiana* populations near strawberry farms for identification of (eventual) loss of genetic variation through genetic assimilation by hybridisation with crops, like Ellstrand (1992) predicted. However, in the end, total genetic variation of these near-farm populations should be compared to proper reference wild populations to assess any loss of variability.

In contrast, the genetic variation of sea beet populations (*Beta vulgaris* ssp. *maritima*) was found to slightly increase, for most parameters, by gene flow from sugar beet as well as red beet/Swiss chard (*Beta vulgaris* ssp. *vulgaris*) (Bartsch *et al.* 1999). Beet may be a special case, since beet cultivars show an equivalent level of genetic diversity as their wild progenitors, which is unusual for a crop/wild species combination (Bartsch *et al.* 1999).

Preliminary analyses of ecological consequences of transgenes in wild populations of *Helianthus annuus* suggest that increased seed production (due to decreased herbivory) in individual plants will lead to an increase in the size of wild populations (Pilson *et al.* 2004 in press). This could increase the frequency of the Bt gene and consequently decrease the population of certain native herbivores; thus a disruptive effect on the herbivore community structure could be expected.

For hybrid populations of transgenic rice (*Oryza sativa*) and the weedy red rice (*Oryza sativa* f. *spontanea*), genetic and agronomic consequences of the transfer of herbicide resistance into weedy red rice populations were examined (Oard *et al.* 2000). The results indicated that populations segregating for the herbicide resistance responded in a site-specific manner with respect to life history and fecundity traits. However, the transfer of the BAR gene is not expected to increase or decrease reproductive success of the weedy hybrids. Therefore, no real ecological or evolutionary effect was likely. On the other hand, the natural hybridisation of non-GM cultivated rice and the endemic Taiwanese taxon, *Oryza rufipogon* ssp. *formosana* has been seen as the cause of the near extinction of this wild rice taxon. Collections of this wild rice showed a decrease of fertility of seed and pollen and a shift toward characters of the cultivated species over the last century (reviewed by Ellstrand *et al.* 1999).

Based on morphological analysis, the wild cotton species *Gossypium darwinii* and *Gossypium tomentosum* were suggested to be at the risk of extinction by hybridisation with the crop *Gossypium hirsutum*. Crop-specific allozyme alleles found in wild

populations, confirmed substantial introgression from the crop into *G. darwinii* (reviewed by Ellstrand *et al.* 1999).

In Switzerland, Rufener Al Mazyad & Ammann (1999) showed that the wild sickle medic (*Medicago falcata*) is overtaken by the cultivated alfalfa (*Medicago sativa*) and the hybrid between *M. sativa* and *M. falcata* (*Medicago x varia*). This was in line with the observations that backcrosses of hybrids to cultivated *M. sativa* were considerably more vigorous than backcrosses to *M. falcata*. In some former populations of *M. falcata*, nowadays only introgressed forms with weakly represented traits of *M. falcata* are found. However, this phenomenon was limited to tetraploid forms of *M. falcata*, since these cross most readily to tetraploid *M. sativa*. A diploid *M. falcata* population occurring in the eastern part of Switzerland was essentially free from introgression by *M. sativa*. Risk of extinction of the wild populations could be increased by genetic engineering and therefore the authors claimed a future ban of genetically modified alfalfa in regions where the wild species is present.

Papa & Gepts (2003 and 2004 in press) argue that asymmetric gene flow (if gene flow from one population, the source, to another, the sink, is higher than in the reverse direction, in this case from crop to wild) will eventually lead to the displacement of alleles of the sink population by alleles of the source populations. In the common bean, *Phaseolus vulgaris*, they found gene flow between domesticated and wild forms to an extent that weedy and some of the wild populations clustered with domesticated populations in a molecular marker-based dendrogram, even though the common bean is a mostly selfing species with typical outcrossing levels of 5% or less. They hypothesized that displacement of genetic diversity of the wild population is occurring in specific genomic regions. This is based on the observation that in the wild populations affected, genomic regions containing domestication genes showed a higher level of diversity than regions lacking them. This is then explained, in their view, by selection against domestication traits in the wild conserving diversity in this part of the genome as opposed to the swamping of the other part of the genome due to asymmetric gene flow from a larger source population. In modern cultivars, the asymmetry of gene flow is expected to become much higher. Pure seed lots are brought in from the original source every one or few years, while hybrids are only used for consumption (or destroyed) and not as seed parents for a consecutive crop. Therefore, the dominant direction of gene flow will further shift from cultivated to wild.

However, most wild populations in their analysis clustered far from the cultivated types, implying that they underwent very little influence from the crop due to their occurrence outside areas of cultivation.

### ***Conclusions***

In general, only few studies have established the environmental and evolutionary effect of hybridisation and introgression. Ellstrand *et al.* (1999) give examples of wild relative taxa that suffer from genetic swamping after hybridisations with conventionally bred crop races. Studies tracking the effect of transgenic races are very scarce. Particularly the potential introgression effects of the integration of traits with fitness value (e.g., apomixis genes or stress tolerance genes), as well as the effects these consecutively could have on wild relatives and the environment have hardly been studied if at all.

### 3. Crop-to-crop gene flow

Gene flow from one crop to the other is mainly a concern of breeders and gene bank curators, who want to keep their varieties/accessions genetically pure (especially seed production crops) or, in the case of organic farmers, free from products of modern biotechnology. Also breeders of transgenic crops will prefer to limit gene flow, because gene escape could result in competing companies or farmers acquiring the unique construct through pollen dispersal, although patents on the constructs used will cover this issue from a legal perspective. Furthermore, transfer of novel traits from one crop to the other could lead to depletions in the quality of conventional and organic crop seed, leading to a change in their performance and marketability (Eastham & Sweet 2002).

Ilardi & Barba (2001) assessed the frequency of spontaneous crossing between homozygous transgenic tomato (*Lycopersicon esculentum*) and untransformed controls. However, in this situation, no transgene flow by pollen was detected; the only transgenic seedlings found were attributable to unintended seed spillage instead of pollination. In contrast, field assessment of gene flow from transgenic to cultivated rice (*Oryza sativa*) showed a significant frequency of pollination of recipient plants with transgenic pollen (Messeguer *et al.* 2001). Even in the predominantly selfing lettuce (*Lactuca sativa*), outcrossing between varieties grown in each other's vicinity was shown up to about 3% (Thompson *et al.* 1958)

In relation to contamination of genetic resources, a notorious case is the Nature report of the occurrence of transgenic sequences in traditional landraces of maize in the Oaxaca region of Mexico (Quist & Chapela 2001). The results were heavily criticised (Christou 2002; Metz & Fütterer 2002; Kaplinsky *et al.* 2002), and as a consequence, the Nature editor admitted that publication of the original paper was unwarranted on the basis of the results presented therein. In a reaction, CIMMYT (International Maize and Wheat Improvement Center) stated that they had taken measures to keep their important genebank collection free of transgene contamination and that in line with that, no transgenes were found upon testing of accessions. As a wind-pollinated, outcrossing species, gene flow into landraces of maize would come as no surprise. However, the whole affair highlighted the danger of premature publication in such a controversial area.

Gene stacking as a consequence of hybridisation between varieties carrying different transgenes is another aspect that is under consideration. In the case of herbicide resistances, this could lead to plant volunteers difficult to control by normal agricultural practice, because they contain resistances against several herbicides at the same time. Triple-resistant oilseed rape volunteers due to cross-pollination have been reported from the Alberta region of Canada (Hall *et al.* 2000). The appearance of such volunteers may lead to adaptation of herbicide treatments depending on the type of crop rotation used, *e.g.*, follow-up by other herbicide tolerant crops like beet. On the other hand, weedy populations can also develop herbicide tolerance under selective



pressure spontaneously, a phenomenon with which the agricultural industry is familiar (Conner *et al.* 2003).

Gene silencing has been demonstrated in a situation where more than one copy of a particular transgene is introduced into a plant and also additional variation in the expression of multiple-copy transgenes has been observed. Therefore it was concluded that multi-copy transgenic lines could be less stable than single copy lines (reviewed in Bavage *et al.* 2002). However, in a study on multi-copy transgenic *Brassica napus*, data on seed set demonstrated that the transgene 'load' of 12 transgene loci did not have a substantial effect on seed production (i.e., a crucial fitness component of the plants). Nevertheless, the experiment shows that the level of transgene expression in the progeny can result in lines with very different levels of transgene expression. This may present a problem where it is desirable to limit the expression of the trait, because the expression of the gene in the progeny from multiple-copy parents cannot be predicted (Bavage *et al.* 2002).

The development of methods to restrict the spread of (introduced) genes to other crops such as various biological and physical barriers to gene flow is of primary concern. The use and width of isolation zones, an area between a (GM) crop and a nearby (non-GM) crop that is either de-vegetated or planted with a non-insect pollinated crop, show varying results (see Eastham & Sweet 2002). A barrier crop is a border of non-GM plants of the same crop surrounding the GM variety that can act as an 'absorber' of the GM pollen. The barrier rows are then destroyed after flowering and before seed fall. This barrier can have a significant influence on gene escape (see Eastham & Sweet 2002). Sheer isolation distance is a good method to prevent gene flow, provided that the capacity of pollen flow should not be underestimated. Timmons *et al.* (1995) examined the movement of pollen grains from oilseed rape fields and assessed their capacity for long-range gene flow using emasculated bait plants. They concluded that oilseed rape has greater capacity for long-range dispersal than had been suggested by small-scale field trials. Also they imply that transgene movement to non-genetically modified fields is likely to follow commercial release.

Biological barriers to prevent gene flow from crop to crop include, for example, the inhibition of flowering to block floral development, genetically engineered male sterility and seed sterility, and inserting the transgene into the chloroplast genome. Since the chloroplasts are maternally inherited, the latter would prevent gene flow through pollen dispersal, but there are exceptions, notably in conifers which have paternal transmission of chloroplasts, but also partially in alfalfa (Stewart & Prakash 1998). Seed sterility has not yet been adopted as a technique for genetic isolation because several aspects of this so-called terminator technology or GURTs (Genetic Use Restriction Technologies) are as yet unreliable (Eastham & Sweet 2002).

### ***Conclusions***

Crop-to-crop gene flow can be expected in virtually every cultivated species that is grown to the reproductive phase, except when strict special measurements are taken (see also Eastham & Sweet 2002). Therefore, prevention of gene flow through artificial barriers, as mentioned above, and physical barriers is recommended. Care should be taken in determining the proper isolation distance; up-scaling of experiments can provide new information and often shows the underestimation of pollen dispersal made in small-scale field trials, as is shown above by Timmons *et al.* (1995).



## 4. Monitoring, the European Union perspective

With the progress in the development of transgenic crops, the concern about the risk of transgene escape has grown (Wolfenbarger & Phifer 2001). Since quite a few transgenic crop races are ready for market introduction, at least according to the opinion of some stakeholders, a regulatory framework has been developed in many countries, and in the EU as well. Recently, the new directive for introduction of GM organisms has been implemented. Directive 2001/18/EC (2001) on the deliberate release into the environment of GM organisms gives the regulatory framework for this, including a set of outlines for the monitoring obligations. Subsequent to this document, a Guidance note for the design of the monitoring plan has been approved. In this Guidance note, a set of principles and practical instructions are given for the actual design of a monitoring plan after the GM plant will have got general consent, and is admitted to the market. Den Nijs & Bartsch (2004 in press) give an overview of this guidance note, seen from the perspective of hybridisation and its consequences for higher plants. According to the EU, the monitoring will have two focuses: the possible effects of the GM crop, identified in the formal Risk Assessment procedure, and any unforeseen effects. Typically, monitoring will start by using a case-specific approach to search for effects mentioned in the RA, this is called “Case specific monitoring”. The second part of monitoring procedure will install a “General surveillance” program, which may contain long term monitoring to be able to find unexpected effects mentioned under the second point. As is stated in paragraph 2.4, for many species baseline data that must give reference values for weighing of the GM effects are missing. Den Nijs & Bartsch.(2004 in press ) emphasise that the availability of such data sets is basic to the monitoring and the subsequent evaluation. The study fields for the data sets, following from the Guidance note instructions, will include the following subjects:

- Biogeography: distribution patterns of taxa concerned have to be studied in order to find areas of co-occurrence of crop and wild relatives,
- Reproductive biology: sexual compatibility of crop and wild relatives and the breeding systems should allow cross-fertilisation,
- Phenology: reproductive ecology must show enough synchrony for cross-fertilisation, distance between the populations should be assessed,
- Demography: demographic characteristics, like weediness, of the wild taxon will help modelling the population development,
- Hybridisation and introgression events in the past,
- Historical and current agronomical and environmental effects.

Part of the basic data, like the above information, has incidentally also been integrated in so-called Botanical Files (Frietema-de Vries 1996; Jacot & Ammann 1999). Screening of herbaria for deposited hybrid specimens to gather information is part of the procedure. However, this data source may lead to underestimations of hybridisation events in the past, although obvious and rare examples of hybrids attract the attention of botanists and have a relatively high chance of being sampled for inclusion in a herbarium collection (Frietema-de Vries 1996). Morphology can be

misleading in some crop-wild relative complexes that involve phylogenetically very close taxa; hybrids will be difficult to detect or easily overlooked. For this problem to be solved, experimental data are needed, which can consist of genetic markers found in the cultivar and/or the wild relative, so that hybrids will be easier to identify.

The use of markers has been suggested as a means of tracing the fate of GM plants as well as introgression events; Green Fluorescent Protein is an *in vivo* marker, which was recently suggested for analysing gene flow (Stewart 1996). Further, it is suggested to make the GM trait itself recognisable by a trait-specific probe or a non-coding GM gene flanking sequence. By PCR-analysis, the trait would then be relatively easy to trace for risk assessment and monitoring (Simonsen 1999).

Den Nijs & Bartsch (2004 *in press*) conclude by stating that monitoring, using the approach prescribed by the Guidance note, will be very costly, laborious and (sometimes) lengthy in time. In practice, this would mean that transgenic varieties are not fit for market release, except perhaps a few widely cultivated and profitable crops, such as maize or soybean. In the case of traits for which escape is regarded as undesirable, production of GM plants in which gene flow is completely prevented seems a better solution to the problem (Kuvshinov *et al.* 2001). For this purpose, Kuvshinov *et al.* (2001) suggested a newly developed method called Recoverable Block of Function (RBF) to overcome unreliability of the previously developed “terminator technology”. This construct will prevent sexual reproduction completely (thus pollen and seed flow is stopped) unless a trigger is applied, which then restores the ability to reproduce.

## 5. Conclusions and Discussion

The general conclusion with respect to the phenomenon of gene flow between crops and wild relatives is that although chances may vary, in many crop-wild relative complexes sooner or later gene flow will occur. Incorporation of crop genes into recipient taxa will occur through further introgression processes after initial hybrid formation. It is necessary to know the consequences of introgression, *i.e.*, the fitness of hybrids and subsequent generations, to assess the persistence of the transgene and its effects on the environment. Fitness of hybrids, in turn, will determine the chances that crop traits will be stably incorporated and further spread in a wild relative. Negative fitness effects of introgression, leading to regional extinction of wild relative population (or complexes) have been reported and make clear that increased weediness is not the only relevant concern.

Although quite some studies on fitness of hybrids, with and without transgenic traits involved, are available (see the reviews by Ellstrand *et al.* (1999) and Eastham & Sweet (2002)) it is clear (see also table 1) that there is still work to be done for many species; especially assessing the extent to which genes, such as those conferring resistance to biotic as well as abiotic stresses, enhance fitness in natural settings as well as improving (methods for) assessment of fitness. The latter should not only focus on scoring relevant fitness traits (such as those related to fecundity) under controlled, *viz.*, contained conditions, but also by monitoring these in realistic field situations. Demographic monitoring could prove a very powerful tool in establishing the life cycle stages that are most relevant to population survival and growth, since it would put the viability and fecundity effects measured under controlled conditions in the proper perspective. This would not only enable to evaluate the pertaining traits for their influence on critical stages during a taxon's life cycle and therefore, for its establishment, survival and spread in the field, but it also may give insight in the effects of changes in patterns of genetic variation, due to introgression processes.

It has become clear that in the end introgression and persistence of (trans)genes in wild populations are trait-specific and depend on numerous aspects. It is evident that, in order to estimate a transgene's capacity to introgress and persist in wild relatives, all elements of the introgression chain need to be considered (Landbo & Jørgensen 1997). Table 1 shows in which crop-wild relative complexes studies have documented the phase of introgression. It is obvious that, relative to the frequency of hybridisation, data on the subsequent introgression is as yet still meagre. Much more data on, for instance, the effects of the transgene insertion site on the introgression process, and the importance of fitness of the intermediate stages (backcrosses) are needed to reach more general insights.

Only limited studies have studied the environmental and evolutionary effects of hybridisation and introgression. Particularly the potential introgression of the use of constructs with fitness value (e.g., apomixis genes –ironically, these are also mentioned as useful for mitigating gene flow (!)- or stress tolerance genes), as well as the effects these consecutively could have on the environment have hardly (or not at

all) been studied. From a practical point of view, there is also the problem that ecological and evolutionary aspects of risk assessment and monitoring studies will frequently involve time axes that encompass many (plant) generations. This is generally conflicting with the economical and political agenda.

Crop-to-crop gene flow can be expected in virtually every cultivated species that is grown to the reproductive phase, because, unless special measurements are taken, there will be no reproductive barriers between the specimens of both taxa (see also Eastham & Sweet 2002). Therefore, prevention of gene flow through artificially engineered barriers, as mentioned above, and physical barriers is recommended. Upscaling of experiments should provide new information and, so far, often shows an underestimation of the extent of pollen dispersal and consequent hybridisation from small-scale field trials, as was shown by Timmons *et al.* (1995).

## 5.1 Knowledge gaps and desiderata

The general conclusion of this report is that knowledge gaps in the field of introgression events and their consequences exist in the following areas:  
outcrossing rates in some crops that have a self-pollination reputation, like lettuce;  
actual introgression of (trans)genes into the genome of the wild relatives;  
fitness of second and later generation hybrids ( $F_2$  and backcrosses); persistence of (trans)genes in wild populations, the ecological and evolutionary effects of this persistence of introgressed genes on the recipient (conspecific) taxa, its competitors as well as its consequences for other trophic levels.

Basic to these points is that in certain crop-wild relative complexes, genetic contacts may have taken place in the conventional situation over longer periods, so that at least in certain areas it is unknown whether wild specimens still keep an “uncontaminated” wild-type genome. Such a situation automatically poses problems for assessing the base line data by which effects from GM cultivars should be weighed. Table 1 shows that for many species baseline data still have to be collected.

It has become clear from the research presented in this report, that introgression and persistence of (trans)genes in wild populations depends on numerous aspects like life history traits of both crop and wild relatives, hybrid fitness (first and later generations), spatial distribution patterns and, last but not least, on the trait engineered into the crop. For instance, Hauser *et al.* (1998a) stressed the need for assessing not only the fitness of  $F_1$  hybrids. They found a fitness barrier in second generation hybrids between *Brassica rapa* and *Brassica napus*, although, judging from the fitness of first generation hybrids, introgression was considered to be likely. The likelihood of hybridisation and introgression may vary between populations and crop and wild type varieties, and will also depend on the crop trait used in crop modification (e.g., Snow *et al.* 1999; Metz *et al.* 1997). Therefore, risk assessment of transgenic plants should cover a range of crop varieties and possible recipient wild populations (Hauser *et al.* 1998a) as well as types of constructs used in modification. Although there are widely acknowledged concerns with transgenic crops (the hybridisation with, and the

subsequent persistence of transgenes in the wild relatives), Table 1 shows the actual research done with GM crops themselves is not overwhelming, and mainly limited to a very few model crops, such as oilseed rape. For example, more studies like the research by Metz *et al.* (1997) are needed to search for possible 'safe' integration sites for the future use of constructs in crops. Up-scaling of experiments would also be recommended to give a better indication of the (trans)gene flow into the surrounding environment; difference in population size may result in different rates of hybridisation and gene flow (Ellstrand *et al.* 1999), e.g., agronomic crop fields normally contain millions of plants, according to the practices of modern industrial agriculture. However, it is noted that experiments with pre-commercial transgenes are inherently difficult to perform due to biosafety and regulatory concerns (Pilson *et al.* 2004 in press). In order to get the essential data, it is necessary to conduct field experiments with the actual transgenic traits themselves. Since competent governmental authorities are reluctant to deliver permits for field experiments, progress is hampered. Also public concern is influencing the research negatively. The rare experimental fields are regularly being destroyed, and plant breeding companies hesitate to co-operate with public domain researchers who would like to study the eventual effects of certain transgenic traits. The use of male sterile plants for field experiments is one solution to eliminate the possibility of transgene escape through pollen, but this, on the other hand, could influence the interpretation of results as is shown by Pilson *et al.* (2004 in press). They argued that the fecundity advantage associated with the Bt transgene in *Helianthus annuus* might have been underestimated by using male-sterile plants.

## 5.2 Towards a European data base

It also has to be stated that ecological and evolutionary effects of (trans)gene flow can not be evaluated and regulated at a national level only, because the whole life history of crop and wild relatives has to be examined, and it is highly likely that regional variation will occur, so that replications are necessary with the biogeography of the pertaining taxon affecting the design of the studies. This is also exemplified by the occurrence of gene flow from wild to crop in seed-producing areas far away from the ultimate cultivation areas but confounding any gene flow from crop to wild occurring there, such as described for carrot and beet under 2.3 above. Moreover, products of modern plant breeding and biotechnology potentially often have a world-wide market, and crops that might not pose a risk in the region of their creation (e.g., because of absence of cross-compatible wild relatives) might give problems in their region of origin with their wild relatives abound. For instance, potatoes bred in NW Europe may end up in their countries of origin in South America. If hybridisation then causes problems (e.g., weed evolution), this cannot be managed by regulating the national farmers in the country of origin alone. What is needed is a European (or better world-wide) biogeographically based data system, where the different and regionally varying relations between crops and the locally occurring wild relatives are put together. Undisputed taxonomical treatment of the pertaining taxa would be a *conditio sine qua non*. Any new risk assessment or post-introduction monitoring study could then be based on up to date, interregionally and internationally comparable base line data.





## 6. Priorities for future research

Based on the surveys presented above, we conclude that satisfying monitoring and evaluation of large scale GM introduction is as yet out of reach, especially with respect to the effects of introgression following initial crop-wild relative hybrid formation. Fitness changes in subsequent hybrid and back-cross generations are an under-explored area. However, certainly with regard to the axiomatic case-by-case approach, more crop species/trait combinations will have to be studied in-depth to be able to come to more general conclusions and decision tools. The knowledge thus gathered should be available in a standardized way, and quantified so that they can serve as inputs for models that can be used in the risk assessment procedure as well as the monitoring phase after market introduction.

Below is a list of research questions to decrease the lacunas in our knowledge. Clearly, these need to be worked out in more detail and translated to a more specific research agenda. Two fields are identified, the first with questions pertaining to the actual process of hybridisation and introgression, and the second concerning approaches to integrate this information in order to study the effects on invasiveness, genetic erosion, and processes in a real, spatially structured agricultural/ecological landscape.

### 6.1 Components of the chain of hybridisation and introgression

WHAT ARE THE ACTUAL RATES OF OUTCROSSING AND INTROGRESSION, ESPECIALLY FOR LESS STUDIED CROPS AND THEIR RELATIVES?

Outcrossing rates and incidence of introgression can be estimated from field data using molecular methods and subsequent statistical analyses. Alternatively, experiments with bait plants can be devised, as well as crossing experiments. This would also involve the development of repeatable and standardized methods to estimate outcrossing and introgression rates, and the information should be quantified such that a link with risk assessment is feasible. In addition, methods for monitoring gene flow that are both reliable and efficient need to be developed.

WHAT ARE THE FITNESS EFFECTS OF INTRODUCED TRAITS UNDER NATURAL CONDITIONS, AND HOW DOES THIS AFFECT TRANSGENE PERSISTENCE AFTER ESCAPE?

Very little data is currently available on how introduced transgenes may alter the ecology of recipient wild species, both in abundance and habitat use. It will be a challenging task for ecologists to predict how a particular transgenic trait may affect wild species after escape. What is at least needed is data on the current ecological niche of recipient species, as well as an identification of sensitive phases in its life cycle using demographic models. Experimental data on plant responses to biotic and a-

biotic factors need to be obtained (in particular if introduced traits concern for instance pathogen resistance, or stress tolerance).

WHAT IS THE FITNESS OF DIFFERENT HYBRIDS, AND ARE SOME PARTS OF THE GENOME MORE LIKELY TO INTROGRESS INTO SURVIVING HYBRID OFFSPRING?

In particular for crosses between different species it should be known what the fitness is of first and later generation hybrids, and whether intense selection and genome rearrangements are likely during this phase. If so, any RA needs to take the variation in outcomes into account, including the effects of different insertion sites of the transgene, which may determine whether it is selected against, or hitchhikes along with nearby other introgressing crop genes.

## **6.2 Integrative and general aspects of GM introductions**

WHAT CAUSES WEEDINESS AND INVASIVENESS IN GENERAL, AND IN CROPS AND THEIR RELATIVES IN PARTICULAR?

Evidence for existing and potential weediness in crop relatives should be collected. In particular transgenic traits that may alter the life history of the recipient plant need careful attention. This includes traits such as seed dormancy, germination requirements, flowering conditions, and breeding system (selfing, apomixis). Experiments are needed to see how such introduced traits may affect plant fitness under field conditions.

As already indicated above, demographic models of recipient species can be helpful in different ways: identification of the sensitive phases in the life cycle of a plant, as a tool to integrate information on different stages into the total lifetime effects, and hence population growth rate, and finally to identify the intensity of natural selection on traits that affect one or more fitness components.

DOES GENETIC SWAMPING OF WILD TYPE NATURAL POPULATIONS BY HYBRIDISATION OCCUR?

Continued directional gene flow from crop (or crop volunteers) to wild relative may lead to an erosion of genetic variation in the species, permanently changing its genetic identity. Such effects need to be studied by analysing traces of gene flow in molecular genetic markers for crop-wild species combinations. This will provide a baseline for the monitoring of future changes connected to the introduction of GM crops.

WHAT ARE THE EFFECTS OF SPATIAL STRUCTURE AND UPSCALING TO AGRICULTURAL POPULATION SIZES?

The translation of the outcome of field trials and experiments under controlled condition is not always straightforward. In the first place, the spatial structure of the agricultural/ecological landscape plays a dominant role, as it determines the distribution of suitable habitats and the distances between crops and wild relatives. In addition, up scaling from field trials to agronomic scales will have a big impact on the

frequency of hybrid formation and hence the likelihood of further spread. This implies that a framework needs to be developed that allows modelling of gene flow and subsequent spread of transgenes, integrating the above information with additional data. Relevant data includes the occurrence of species (for instance using inventory databases of plants and insects), abiotic factors, natural barriers to gene flow, etc. Geographic Information Systems (GIS) could provide the necessary tools.



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## Appendix

Crop	Wild/weedy relative	2.1 Gene flow		2.2 Fitness hybrids		2.3 Introgression		2.4 Ecological/Evolutionary effects
		Gene flow trad.	Gene flow GM	Fitness hybrids trad.	Fitness hybrids GM	Fitness backcrosses trad.	Fitness backcrosses GM	
<b>Agrostis stolonifera</b>	Agrostis canina	None (9)						
	Agrostis castellana	0.0015% (9)						
	Agrostis gigantea	None (9)						
	Agrostis capillaris	0.044% (9)						
<b>Amaranthus hybridus</b>	Amaranthus rudis	intercrossable (104)						
<b>Beta vulgaris</b>	Beta vulgaris ssp. maritima	detected indirectly (7,29)			virus resistance by controlled cross (27)			increase in genetic diversity (7, 29)
	Beta vulgaris	detected indirectly (107)	detected indirectly (29, 107)	0.2-5.9% on weed beet in field (29)				
	Beta macrocarpa	introgression detected in 13 of 594 (2%) of wild (29)						
<b>Brassica napus</b>	Brassica napus (crop)			0.0156% at 200 m, 0.0038% at 400 m (89); 50% within 3m from individual then along negative exponential of distance: better estimator than pollen dispersal from whole plot (59); higher than expected from large fields (102)	high stearate later germination, seedling lower biomass, <, sometimes> (63), dormant / high-laurate equivalent better (seedling growth rate and sometimes>dormancy, 63) than control (64)	variability in GUS expression unrelated to copy number in segregating population (8)	8-year survival of escapes on road verges (78)	

Crop	Wild/weedy relative	2.1 Gene flow		2.2 Fitness hybrids		2.3 Introgression		2.4 Ecological/Evolutionary effects	
		Gene flow trad.	Gene flow GM	Fitness hybrids trad.	Fitness hybrids GM	Fitness backcrosses trad.	Fitness backcrosses GM	Persistence (trans)gene	
Brassica rapa	0.4-1.5% outside crop field (29), 1 in 505 near crop fields (potential habitats detected by GIS) (112); 13-93% depending on ratio of species in field, Br SI (29)	controlled crosses GFP (39), 9%(of 482 offspring) HT in 1:7 ratio of species in field (29)	<2% seedlings survived in field (29), Bn>F1 hybrids>Br in fitness estimate (45), hybrids non-dormant like Bn (58)	Bt resistance to herbivory in controlled crosses (39), BrXBn high-laurate appearing fitter during seedling development (~Br) than high-stearate in greenhouse, but dormancy<Br (overriding usual maternal effects) (64)	F2-backcrosses<Bn, Br in fitness estimate, some offspring as fit (44), BC1 more dormant than F1 (58)	with selection 5,5% of backcrosses transgenic (65), BC3 50% in BC3 in glasshouse (93), HT in 10% of controlled BC3+4, variability like Br in glasshouse ascribed to integration in A or C genome (70)	15-34% backcrosses GFP transgenic (39), HT 50% in BC3 in glasshouse (93), HT in 10% of controlled BC3+4, variability like Br in glasshouse ascribed to integration in A or C genome (70)		
Brassica oleracea	none in field situation (112), poorly intercrossable, none in field(29)		pollen reduced fertility (29)		F2 poorly germinating (29)				
Brassica juncea	intercrossable, 3% field, other way around less (29)	intercrossable, 3% field, other way around less (29)	pollen fertility 0-29% (29)		pollen fertility improved to 24-90% (29)		Bn RAPDs 19-93%, HT transgene 52% in BC1 (29)		
Brassica nigra	1 of 1000 controlled, 1 of 100 reciprocally, none in co-cultivation (29)	none in co-cultivation (29)							
Raphanus raphanistrum	45 per male-sterile (29)	none (84), 10-7 to 3.10-5 (14), 6.10-5 to 2.10-3 (Rr SI, 20), none over 5 years in natural setting (29)	dormancy Bn<F1<Rr (13), less than 1 Rr BC1 per BnxRr plant improving in subsequent generations (29)	F1 1.8(max13) seed/plant, fitness relative to Rr 0.05 (max 0.4)% (20); F1(BnxRr) 1.1 seed/plant, 28.6% seeding (14); field: BC1(F1xRr) 0.12 seed/100 flowers-0.78 seed/plant-male fertility 8.7%-female 1.4/100-11/plant (15)	BC1 female fertility 10xF1, BC2 approaching Rr, BC4 25.4% Rr chromosome#2n=18 (14); BC2(BC1xRr) 7.9/100-229.3/plant- chromosome# down to 10.5% Rr#:2n=18 (15); F2-BC1 15.2 seeds/plant, fitness relative to Rr 0.4(max2)% (29)	BC1 female fertility 10xF1, BC2 approaching Rr, BC4 25.4% Rr chromosome#2n=18 (14); BC2(BC1xRr) 7.9/100-229.3/plant- chromosome# down to 10.5% Rr#:2n=18 (15); F2-BC1 15.2 seeds/plant, fitness relative to Rr 0.4(max2)% (29)	BC4 from BnxRr HT and close to Rr in BC4 morphology (14); BC1(F1xRr) male fertility 8.7% (14); BC2(BC1xRr) female 1.4/100-11/plant, BC2(BC1xRr) 57.2% HT-chromosome# down to 10.5% Rr#:2n=18 (15), no stable integration HT (29)	scenario: max 73 F1/ha>2000 F3-BC2(0.4x10-6 total Rr seed production, but under HT selection 71%) (20)	

Crop	Wild/weedy relative	2.1 Gene flow		2.2 Fitness hybrids		2.3 Introgression		2.4 Ecological/Evolutionary effects
		Gene flow trad.	Gene flow GM	Fitness hybrids trad.	Fitness hybrids GM	Fitness backcrosses trad.	Fitness backcrosses GM	
	Hirschfeldia incana(syn. Brassica adpressa)	fertilisation efficiency relative to intra-species 15% HixBn, 1.3% reciprocally, in controlled crosses, 26 seeds per male-sterile Bn plant in field (61)	fertilisation efficiency relative to intra-species 15% HixBn, 1.3% reciprocally, in controlled crosses, 1 seed per Hi plant (61); 0.6 per plant over 3 years in field (29)	F1xHi 0.5 seed per plant (61); hybrid > Hi, but fertility 0.2 seeds/plant (29)		no viable seed in BC5 (29)		
	Sinapis arvensis	none in >7500 Sa seeds (29); 0 in 3.8million seeds from Sa, 6 from 50000 male-sterile Bn flowers in field (29); 3.7 per 100 flowers by hand pollination/in vitro culture, 0.18 from 100 male-sterile Bn flowers in field, reciprocally 0 (29); 0 from 6420 and 1 from 1127 hand pollinations (0.0015% seeds) on Sa, 1 0-0.0049% on Bn by hand pollination, 0 in 10.000 seedlings from 26 Sa in field (72)	fertility very low (72)			no backcrosses possible (72)		
<b>Brassica rapa</b>	Brassica rapa	gene flow unhindered between vegetable and wild form (31)		low dormancy/mortality crop < F1 < high dormancy/mortality wild, closest to female parent (1)			chance for establishment inferred from fitness data (1)	
<b>Coffea arabica</b>	Coffea liberica	introgressions detected by AFLP (80)						



Hybridisation and introgression

Crop	Wild/weedy relative	2.1 Gene flow		2.2 Fitness hybrids			2.3 Introgression			2.4 Ecological/Evolutionary effects
		Gene flow trad.	Gene flow GM	Fitness hybrids trad.	Fitness hybrids GM	Fitness backcrosses trad.	Fitness backcrosses GM	Persistence (trans)gene		
<b>Cucumis melo</b>	Cucumis melo (crop)	no difference between traditional and GM (46)	no difference between trad. and GM, sometimes transgene inactivation (46)							
<b>Cucurbita pepo</b>	Cucurbita texana	5%, up to 1300 m by solitary bee species (51)			no fitness difference between trad. and GM-virus resistance, hybrids more virus-resistant (97)					
<b>Daucus carota ssp. sativus</b>	Daucus carota ssp. carota	gene flow probable from bolters, both purely cv. and wild>crop hybrid from seed production (42)		wild>>hybrid>cultivar in frost sensitivity (41)						
<b>Fragaria x ananassa</b>	Fragaria virginiana	cultivar AFLPs detected in wild populations (109,110)							persistence of outdated cultivar AFLPs detected in wild populations (109,110)	
<b>Glycine max</b>	Glycine soja	artificial hybrid dead before anthesis (29) intermediates near crop fields, hybrid taxon described as G. gracilis (31)								
<b>Gossypium barbadense</b>	Gossypium hirsutum	crop alleles detected in wild (31)								
<b>Gossypium hirsutum</b>	Gossypium barbadense	crop alleles detected in wild (31)								
	Gossypium darwinii	substantial # crop alleles in wild (31)								substantial # crop alleles in wild, risk of extinction suggested (31)
	Gossypium tomentosum	hybridisation suggested morphologically, no markers found (31)								risk of extinction suggested (31)

Crop	Wild/weedy relative	2.1 Gene flow		2.2 Fitness hybrids			2.3 Introgression			2.4 Ecological/Evolutionary effects
		Gene flow trad.	Gene flow GM	Fitness hybrids trad.	Fitness hybrids GM	Fitness hybrids	Fitness backcrosses trad.	Fitness backcrosses GM	Persistence (trans)gene	
	<i>Gossypium mustelinum</i>	limited # crop alleles in wild (31)								
<b>Helianthus annuus</b>	<i>Helianthus petiolaris</i>	0.006 to 0.026 cultivar-specific AFLP in wild populations (85)								variability in presence of cultivar-specific AFLPs in wild populations (85)
	<i>Helianthus annuus</i>	27% at 3m, up to 1000 m (3, 31), 42% at crop margin (111)		hybrid seeds larger and under larger herbivory (2), hybrids less dormancy, similar fecundity, more rust-resistant, but all variable, than wild (94)	no fitness effect of transgenic oxalate oxidase white mold resistance in field (12)				reduced herbivory by Bt under field conditions (79, 95)	persistence of crop alleles over 5 generations (111)
<b>Hordeum vulgare</b>	<i>Hordeum vulgare</i> (crop)	99% selfer, but cross up to 60 m possible, isolation distance 1 m sufficient (29)	<2% on male-sterile plants in field, HT volunteers occurring (29); 100% at 1 m, 3% at 50 m on male-sterile, 0-7% on male-fertile at 1 m (87)							
	<i>Hordeum vulgare</i> ssp. spontaneum	intermediates near crop fields (31)								
	<i>Hordeum jubatum</i>	intercrossable (29)		vigorous progeny infertile (29)						
	<i>Hordeum secalinum</i>	intercrossable (29)		progeny slowly growing infertile (29)						
	<i>Hordeum marinum</i>	progeny haploids of Hma (29)								
	<i>Hordeum murinum</i>	progeny (di)haploids of Hmu dead before anthesis (29)		progeny dead before anthesis (29)						

## Hybridisation and introgression

Crop	Wild/weedy relative	2.1 Gene flow		2.2 Fitness hybrids		2.3 Introgression		2.4 Ecological/Evolutionary effects
		Gene flow trad.	Gene flow GM	Fitness hybrids trad.	Fitness hybrids GM	Fitness backcrosses trad.	Fitness backcrosses GM	
<b>Lactuca sativa</b>	Lactuca sativa (crop)	up to 3% (100)						
	Lactuca serriola	cross-compatible in controlled crosses (23)						
<b>Lolium perenne</b>	Lolium perenne	small populations might be swamped by immigrant pollen, even if not directly downwind of the source (modelled by 33), much variation in pollen dispersal by wind not well described by Bateman's equations (34), turbulence needed in addition to wind direction in pollen dispersal models (35)						
	Lycopersicon pimpinellifolium	cross-compatible in controlled crosses (21)						
<b>Lycopersicon esculentum</b>	Lycopersicon esculentum (crop)	no pollen flow detected in field experiment, only seed lot contamination (47)						
	Malus domestica	intercrossable, level in field unclear (82)						
<b>Medicago sativa</b>	Medicago falcata	extensive (88)						swamping of wild populations (88)
	Medicago sativa	gene flow through seeds implied by mtDNA markers (73)						
<b>Oryza glaberrima</b>	Oryza glaberrima	intermediates near crop fields (31)						

Crop	Wild/weedy relative	2.1 Gene flow		2.2 Fitness hybrids		2.3 Introgression			2.4 Ecological/Evolutionary effects
		Gene flow trad.	Gene flow GM	Fitness hybrids trad.	Fitness hybrids GM	Fitness hybrids GM	Fitness backcrosses trad.	Fitness backcrosses GM	
	<i>Oryza sativa</i>	intercrossable (31)		F1 sterile (31)					
	<i>Oryza barthii</i>	intermediates near crop fields (31)							
	<i>Oryza longistamina</i>	intermediates near crop fields (31)							
<b>Oryza sativa</b>	<i>Oryza sativa</i> (crop)	intermediates near crop fields (31)	0.01%, up to 0.53% downwind (68)						
	<i>Oryza sativa</i> f. spontanea(red, weedy)	1-52% in mixed stands depending on cv. (31)		F1 heterotic (31)	no significant differences in fitness (74)			introgression of pigmentation allele in a few seasons (31)	
	<i>Oryza nivara</i>	intermediates near crop fields (31)							near extinction by swamping (31)
	<i>Oryza rufipogon</i> (perennis)	296 out of 23776, maximum 43.2 m, depending on wind direction (96)							near extinction by swamping (31)
<b>Phaseolus vulgaris</b>	<i>Phaseolus aborigineus</i>	intermediates near crop fields not ubiquitous (31)							asymmetric gene flow influencing weedy hybrid populations (76,77)
	<i>Phaseolus mexicanus</i>	intermediates near crop fields not ubiquitous (31)							
<b>Populus trichocarpa x deltoides</b>	<i>Populus trichocarpa</i>	0.7-1.9% max. 3.8% within 500 m from plantation (28)		hybrids as fit as wild (28)					
<b>Prunus domestica</b>	<i>Prunus spinosa</i>	interfertile (29)							
<b>Raphanus sativus</b>	<i>Raphanus sativus</i>	potential hybridisation level increased with distance contrary to existing pollen flow theory (53,54)		hybrids more fit than wild (55)					

## Hybridisation and introgression

Crop	Wild/weedy relative	2.1 Gene flow		2.2 Fitness hybrids		2.3 Introgression		2.4 Ecological/Evolutionary effects
		Gene flow trad.	Gene flow GM	Fitness hybrids trad.	Fitness hybrids GM	Fitness backcrosses trad.	Fitness backcrosses GM	
	Raphanus raphanistrum							F1 hybrids not at disadvantage with pollinator visits when at low frequencies or in presence of bumble bees (60)
<b>Rubus idaeus</b>	Rubus idaeus	L gene (fruit size) not, s gene (spines) 0.004 in wild near cultivation, not in remote areas (66)						
<b>Solanum tuberosum</b>	Solanum tuberosum (crop)		1.14% in crop to 0% at 4.5-6 m (29); 24% next to crop, 2% at 3 m, 0.017% at 10 m to 0 at 20 m (29); up to 1000 m by pollen beetle in New Zealand (29)					
	Solanum nigrum	0 by hand pollination (29)	0 in 8148 seedlings from 77 plants in field (29)	F1 seeds from male-sterile Sn not viable, progeny from embryo rescue sterile (29)				
	Solanum dulcamara	0 by hand pollination (29)	0 in 1102 seedlings from 63 plants in field (29)					
<b>Sorghum bicolor</b>	Sorghum halepense	hybridisation detected by isozymes 0.5-100m varying with location and year (5)		no difference in fitness parameters with wild parent (6)				enhanced weediness by hybridisation implicated (31)
	Sorghum bicolor	intermediates near crop fields (31)						

Crop	2.1 Gene flow		2.2 Fitness hybrids		2.3 Introgression		2.4 Ecological/Evolutionary effects
	Wild/weedy relative	Gene flow trad. Gene flow GM	Fitness hybrids trad.	Fitness hybrids GM	Fitness backcrosses trad. backcrosses GM	Persistence (trans)gene	
	Sorghum propinquum	intermediates near crop fields (31)					hybridisation implicated in origin of weedy <i>S. alatum</i> (31)
<b>Triticum aestivum</b>	Hordeum marinum s.str. Huds.	1 of 88 (from 2 pops) with SSR and RAPD, but no isozyme markers from wheat (29, 37)					
	<i>Elymus caninus</i> L.	none (37)					
	<i>Triticum turgidum</i>	>98% selfer, hybrids spontaneously in field (29)	hybrids mostly sterile (31)				
	<b>Secale cereale</b>	artificial hybrid, Triticale, not detected in field (29)					
	<i>Aegilops cylindrica</i> Host.	none in greenhouse, 85 of 2400 in field (RAPD+SSR) varying with population (36); hybrids in greenhouse (29)	2 in field research plot (29)	F1 female fertility 2% in greenhouse (29); hybrids mostly sterile (31)	BC1 12 seeds in 13 from 5 plants germinating partly fertile (36); BC1 and BC2 (female fertility 37%) formed in field with high germination rate and partial self-fertility (29); TaXAc F1 only female fertile, BC1 with 1.8% male/4.4% female fertility, BC2 8.9% male/18% female/6.9% self fertility, sometimes alien chromosomes kept (108)	6 in 7 BC1 seedlings from 2 F1 plants HT (29)	

## Hybridisation and introgression

Crop	Wild/weedy relative	2.1 Gene flow		2.2 Fitness hybrids		2.3 Introgression			2.4 Ecological/Evolutionary effects	
		Gene flow trad.	Gene flow GM	Fitness trad.	Fitness hybrids GM	Fitness hybrids	Fitness backcrosses trad.	Fitness backcrosses GM		Persistence (trans)gene
<b>Zea mays mays</b>	Zea mays ssp. mexicana	cross-compatible (29); intermediates near crop fields, crop alleles extremely low (31)							evidence for introgression under debate (29, 31)	
	Zea luxurians	crop alleles extremely low (31)								
	Zea mays ssp. mays (crop)	down to 1% at 20-40 m, 0.2% at 500-800 m (standard isolation distance 200 m) (29)								
	Zea diploperennis	crop alleles extremely low (31)								
	Zea perennis	not compatible (31)								
<b>Abbreviations: GIS = Geographical information Systems; HT = Herbicide Tolerance; SI = self incompatible</b>										