

Chapter 12

Vertical (trans)gene flow: Implications for crop diversity and wild relatives

DAVID QUIST

NORWEGIAN INSTITUTE OF GENE ECOLOGY (GENØK), TROMSØ, NORWAY

The purpose of this chapter is to present an overview of the potential evolutionary consequences of (trans)gene flow, focusing on crop plants. From a scientific standpoint, the challenge is to determine and gather all of the relevant scientific knowledge possible, identify uncertainties and known gaps of knowledge, and use this information to design a context-specific framework to guide the safe use of a particular technology. Likewise, understanding and minimizing the potential safety impacts of GMO crops requires identifying the relevant issues and information – not only genetic and biological information, but also socio-cultural and legal dimensions as well. In this case, I will introduce rudimentary concepts of gene flow, discuss the current state of knowledge, assumptions and future needs in biosafety research. The objective is to contextualize the scientific issues to help understand the issues for developing a sound scientific assessment of the potential implications of vertical transgene flow for crop biodiversity, weed and target resistance evolution, and food security. From this, a series of critical questions and needs emerge, and can be added to discussion and decision making within the realm of a particular country, crop, and/or policy regime. Other emerging issues, such as the impacts on human health and environment, are discussed in Chapters 14 and 10, respectively, and are outside the scope of this chapter. Note that this chapter is intended to give only a basic introduction to the subject, yet provides references to key literature in the field for further reading (for extensive reviews on the subject, see Ellstrand et al. 1999; Ellstrand 2001; Eastham & Sweet 2002; Gepts & Papa 2003; Messeguer 2003; Snow et al. 2004).

In Section 1, I will introduce the basic concepts in biology of gene flow. Section 2 will be dedicated to discussing the potential evolutionary significance of transgene flow from a) crop to wild relative, b) crop to landrace, and c) crop to crop, each of which have their own set of emergent socio-cultural, political and economic considerations. These will be illustrated by recent research and actual transgene flow events. In Section 3, I will discuss some of the means of tracking transgenes. Section 4 contains a discussion of some critical gaps in scientific understanding and uncertainties that should be communicated to policy makers, and the general public, for making informed decisions on the safety of transgenic crops. In the fifth and final section, I discuss some questions that may be useful in consideration of policy and risk assessment concerning GMOs with respect to crop biodiversity and food security issues.

1. Overview of vertical gene transfer (gene flow)

1.1 What is gene flow?

Gene flow is the movement of genes from one population to another, conferring new traits – the biophysical characteristics of the organism – to individuals of the recipient population. This happens by *cross-pollination* (also called *hybridization*), that is, the pollination of members of one population or genetic pool with that of another. The outcrossing of genes is said to be ‘vertical’ as the genetic information is passed ‘down’ from parents to offspring. This is contrasted with *horizontal gene transfer* (discussed in Chapter 13), where the acquisition of genes is passed over, i.e. ‘horizontally’, from one organism to another by means other than inheritance. Vertical gene flow often results in *introgression*, the establishment of *alleles* (gene variants), or wholly new genes (as is the case with transgenes) in the recipient population.

Therefore, vertical gene flow is restricted to organisms that can mate with one another and make offspring. In the case of crop plants, which are domesticated forms of wild plants, a high degree of compatibility can therefore exist between the crop and wild and weedy relatives. Gene flow can be from crop to crop (or landrace), from crop to wild relative, and even from wild relative to crop plant. Gene flow has been a natural, and in some cases desirable, part of evolution and speciation in flowering plants (Anderson 1961; Reiseberg & Wendel 1993; Ellstrand et al. 1999). Thus, gene flow per se is not the main concern, but rather the *types of genes*, and the level of genetic heterogeneity or homogeneity (genetic diversity) that is spread through gene flow and its effect on recipient populations, are the relevant issues. Whether flow of new genes or gene variants results in a change in *fitness*, i.e. the ability of the organism to survive *and* produce viable offspring (either positively or negatively), has been a central focus in population biology. It should be clearly pointed out that considerations of gene flow discussed here are not unique to transgenic crop varieties, but are relevant for all commercial crops. The introduction of wholly new genomic identities into recipient populations from commercial traits should be equally scrutinized, but is outside the scope of this chapter.

If commercial crops have been exchanging genes with related species for some time, why are transgenic varieties of particular concern? With transgenics, completely novel traits are passed on that could dramatically affect the fitness of individuals receiving the given gene in a population. Thus, the commercialization of transgenic plants has sparked widespread interest in the potential evolutionary significance of transgene flow. The central question is how transgene introgression may impact fitness in the new transgenic hybrids, and consequently, the significance for maintaining important crop genetic diversity for future crop breeding.

1.2 Under what conditions does gene flow occur?

Hybridization and subsequent gene flow depend on a number of biological and ecological conditions. First, the sexually compatible plants need to be growing within sufficient pollen or seed dispersal range of the transgenic crop. In many cases, there is no overlap between crops and wild/landrace relatives, and they do not pose a concern, yet crop to crop gene flow often is a concern. The possible dispersal range of reproductive propagules (i.e. pollen) is dependent on many different climatic (including wind, humidity, temperature, etc.) and biological factors (height of plant, size of propagule, natural outcrossing rates, etc.), but human dispersal can also broaden this range. Second, in order for gene flow to occur, there must be an overlap in *phenology* (flowering and fertility times) between the transgenic crop and recipient population. Flowering times may be affected by ecological and or biological factors in some circumstances, leading to partial or total reproductive isolation among neighbouring populations. Third, any mating between a transgenic crop and a landrace or wild relative must produce fertile and viable offspring. Reproductive barriers to introgression are strong, especially where *ploidy number* (genomic copy number) differs between domesticated and wild crop relatives (Jenczewski et al. 2003). This may only occur in limited scenarios. Plants that normally are only self-compatible, i.e. have the capacity to only mate with itself, also represent a type of reproductive barrier. Fourth, the offspring of the new transgenic-hybrid plant must also be viable and fertile to some extent, and a lack of survivors means that any potential gene flow would cease at this point. Yet even a low level of fertility can lead to fully viable populations in subsequent generations, as would be the case with *backcrossing* (mating ‘back’ or again, with the parent population) into the wild progenitor populations.

When these four conditions are met, transgene flow is likely. In some of these cases, offspring will have reduced fitness, or produce sterile (unviable) seeds. In other cases they will have improved vigour (Singh et al. 1995; Hauser et al. 1998) and fitness, yet the advantages may reduce or reverse over time. Thus, there must be a minimum level of fertility in order for the

recipient population to maintain the transgene(s) and survive to the next generation. Lastly, it should be mentioned that the dispersal of seeds themselves can also be an agent of gene flow. The movement of seeds can occur in a range of ways, mostly by human activities, such as transportation (Figure 12.1), or by wind or wild animals.



Figure 12.1. A maize plant growing on the side of the highway outside Guadalajara, Mexico. This plant presumably arrived as a seed fallen from a transport truck. (Photo: D. Quist, 2002)

1.3 In what species or kinds of crops could transgene flow occur?

Almost all of the world's most important crop plants are known to hybridize with wild relatives. At least 44 cultivated crops have demonstrated the capacity for hybridization with wild and weedy relatives, including 12 of the 13 most widely cultivated crops (Ellstrand et al. 1999), and 11 of the 20 most important US crops, including sunflower, radish, sorghum, canola, squash, rice, wheat, sugar beet, lettuce, poplar, strawberry, and bentgrass (Ellstrand 2003). As discussed, gene flow to wild relatives and landraces will depend on the availability of such species near the area of cultivation (Messeguer 2003). Crop to crop gene transfer often occurs where transgenic and non-transgenic crops are planted in close proximity. Many of these crop plants are primarily

outcrossing species, including maize, canola (rapeseed), tomato, sorghum, wheat, sugar beet, alfalfa, cucumber, radish, and strawberries (NRC 2000).

2. (Trans)gene flow and its potential evolutionary consequences

So why might changes in plant fitness (its ability to survive and reproduce) resulting from transgene flow be significant? Effects on fitness are largely dependent on the nature of the genetically engineered traits, and the external and internal factors that influence their expression (Gepts & Papa 2003; Jenczewski et al. 2003). As approximately 97% of all transgenic crops involve insect resistance or herbicide resistance, these are the main traits under consideration (yet the history of unintended transgene flow events, and the coming generation of plant-made pharmaceuticals are perhaps signs of things to come). In the case of insect resistance transgenes, levels of pest pressure in wild or landrace populations may be lower compared to crop populations, reducing the selective value of the trait. Herbicide resistance genes might exhibit an energetic cost on the hybridized plant that would have no value if the herbicide is not applied (but alternatively, great value if it is). In the case of stress-tolerant transgenic crops (drought tolerance, salt tolerance, etc.), with traits that allow survival in a broader range of ecological conditions, hybridization is likely to increase fitness and invasiveness.

Hence, whether transgenes from a source population will establish in wild or landrace sink populations will depend on a number of independent and interrelated factors – genetic, ecological and even human management variables. Identifying the most important components to survival is not straightforward, and must be considered within the ecology of transgenic hybrids. Variation in fitness is also likely across hybrid generations. With such little knowledge on the behaviour of transgenes in unintended and new genomic and ecological backgrounds, prediction of real-world effects is particularly challenging.

One principal concern of transgene flow is the loss of potentially useful crop genetic diversity in the recipient population (whether other crops, landraces or wild relatives). *Outbreeding depression* (the reduction of fitness from hybridization) can lead to a decrease in allelic diversity by extinction of members of a diverse gene pool that are less adapted to survive because of the particular introgressed transgenic trait. This is loss of diversity through negative selection. On the other hand, when transgene hybrids have an increased fitness, and can survive into the next generation, *genetic assimilation* (loss of unique genetic identity through continual hybridization and backcrossing) will have a homogenizing affect on the recipient population, also leading to a less diverse gene pool. Thus, both instances can have negative effects on genetic diversity. The magnitude of these selective forces within the new genomic and ecological background of the recipient population will largely determine the rate of evolutionary change in the recipient population (Gepts & Papa 2003).

So how can we predict the outcomes of transgene flows on a recipient population? Population matrix models have been suggested as useful ways to estimate this risk (Parker & Kareiva 1996; Bullock 1999). However, the magnitude and evidence of effects is idiosyncratic, and may take years to develop (Ellstrand & Hoffman 1990). Few direct studies have been conducted to measure the fitness effects of transgenes in wild populations (Linder 1998; Linder et al. 1998; Snow et al. 2001; Spencer & Snow 2001; Gueritane et al. 2002; Snow et al. 2003). Of these, many were conducted under ideal agricultural conditions, where water and nutrients were not limiting, and interspecific competition was low, rather than stress conditions often faced by low- or unmanaged populations. Further, many studies seeking to understand persistence of transgenes in natural populations have only studied the first hybrid generation. Some investigators have questioned the value of such estimates in early hybrid generations (Linder et al. 1998), as variation in fitness

may occur across generations due to recombination and selection (Hauser et al. 1998). Models to quantify such changes over subsequent hybrid generations have been useful to help predict potential outcomes of such events through time (Lavigne et al. 1998).

A useful model to study survivorship after gene flow is *migration-selection balance*. This model demonstrates (Lenormand 2002) that in crop to crop, or crop to wild gene flow, even *negatively selected traits* (traits that decrease the plants' ability to survive) are still likely to be maintained (in balance) in the recipient population. Whether this allele is maintained or not depends on the level of gene flow to the population. If there are sufficient rates of gene flow of the negative selected allele, a threshold value will be reached, leading to *fixation* (permanent maintenance of the allele in the population). In this case, the sub-optimal allele would predominate purely by magnitude of gene flow coming into the population.

Given the importance of introgression for the evolution of land plants, and the ubiquity of gene flow between crops and wild relatives, the impacts on native genetic diversity is a broad concern (NRC 2000; Pilson & Prendeville 2004; Snow et al. 2004). Some investigators downplay these risks, assuming that if transgene flow produced offspring of low fitness, the transgene would not survive in the population at all. Yet, research contradicts this assertion. Theoretical studies suggest that introgression rates of genes from one population to another can be quite rapid even when the fitness advantage is small (Barton & Dracup 2000), or when there is a high frequency of transgressive hybrids (Reiseberg & Wendel 1993). A modelling study conducted by Haygood et al. (2003) demonstrated that crop alleles can be rapidly fixed in a recipient population when the migration frequency exceeds the selection threshold, even when they have a negative impact on fitness. Their study expands on how *demographic swamping* (reduced fitness in the hybrid's offspring populations) can facilitate genetic assimilation just where high rates of gene flow occur from agricultural populations. In this situation, gene flow that reduces fitness will become stable in the population when the migration rate of the alleles exceeds the level of selection, leading to reduced population size and perhaps local extinction. Further, extinction through hybridization is a valid concern not only when it involves transgenic plants, but in any situation of non-native biological or genetic invasions (see Chapter 11 on invasives) where hybridization may increase a plant's invasiveness (Ellstrand & Schierenbeck 2000).

2.1 Types of transgene flows and their implications

With the now decade-long history of GMO commercialization, the world has already witnessed a number of cases of transgene flow, from crop to wild relatives, crop to landrace and crop to crop. Within each type of transgene flow, a host of environmental, agronomic, cultural, and intellectual property concerns emerge in conjunction with the biological and evolutionary considerations of gene flow. While research has made some progress, there is still much to be learned.

2.1.1 Gene flow from crops to wild and weedy relatives

Transgene flow, generally regarded as undesirable and hence often regarded as 'transgenic contamination', presents a number of management challenges with the formation of transgenic hybrids in sexually compatible weed species (Darmency 1994; Snow & Palma 1997). Hybridization may give distinct selective advantage over non-hybrids in a population, particularly where certain herbicides are used to control these weeds – and can allow the hybrids to become more invasive in natural and agricultural habitats (Ellstrand 2003). Increased weediness of some wild relatives also augments their invasive potential into new environments whereas resistance to insect damage is inherited from insect-resistant crops. Gene flow from crops to wild relatives has been linked to the evolution of weediness in seven out of the thirteen most important crop plants (Ellstrand et al. 1999).

A good example of transgene flow between a crop and its wild relative is that of transgenic oilseed rape (also called canola) *Brassica napus*, and its wild relative *B. rapa*. Early research suggested that hybrids between oilseed rape and the weedy *B. rapa*, would be minimal, due to gene flow barriers and low survival (Crawley et al. 1993). However, later research by Mikkelsen et al. (1996) and Hall et al. (2000) have shown wide dispersal of herbicide tolerance genes in weedy *B. napus*. Gene flow has subsequently been shown to persist for many years (Pessel et al. 2001; Simard et al. 2002). This has led to a number of distinct challenges for weed management near agricultural lands.

Another example involves the escape of transgenes from glyphosate-resistant (a herbicide) bentgrass (*Agrostis stolonifera*) in the United States. Reichman et al. (2006) detected transgenic hybrids with weedy *Agrostis species* some 3.8 km downwind of transgenic field trials, in federally-protected grassland. The ecological consequences of such outcrossings are uncertain, yet any decrease in genetic diversity would lead to a change in community structure with the introgressed regions. As a result, in 2007 a federal judge ordered a temporary halt in new approvals of GM field trials, citing an inadequate environmental review of the potential environmental impacts.¹ The ruling requires that future GM trials in the US must undergo more rigorous environmental reviews.

Whether or not any resulting gene flow has an evolutionarily significant effect on wild and weedy relatives must be tested carefully. Few studies have directly addressed crop to wild transgene flow in the field (Linder & Schmitt 1995; Linder et al. 1998; Bartsch et al. 1999; Spencer & Snow 2001; Gueritain et al. 2002; Snow et al. 2003). Researching the impacts is difficult, as the selective value of a transgene in a wild population may be different within its ecological and biological context, where a host of factors (including epistasis, genetic drift, etc.) may influence the magnitude of evolutionary impact. Nonetheless, cases such as with the aforementioned creeping bentgrass signal the need for more intensive research in this area.

2.1.2 Crop to landrace gene flow

Gene flow between modern crops and *landraces* – the genetically diverse domesticated, local, farmer-selected cultivars – has been an area of concern since the early inception of modern plant breeding. Many landraces are still being cultivated within their areas of origin, and hence, local farmers play an important role in the maintenance of in situ diversity and conservation (Gepts & Papa 2003). Landraces act as important sources of genetic diversity – the genetic stock that plant breeders must rely on for future crop improvement. For this reason protection of this diversity has been a concern of international crop research centres, international agencies, and national governments alike. The loss of this diversity involves not only food security considerations, but also cultural notions of patrimony and locally-derived genetic resources.

Centres of crop origin and diversification therefore both play crucial roles for future crop breeding. Figure 12.2 details some centres of origin for some of the world's most important food crops.

¹http://www.centerforfoodsafety.org/GTBC_DecisionPR_2_7_07.cfm accessed 10 February 2007



Figure 12.2. Centres of origin and diversification for major crops. Other geographic areas may as well contain important sources of genetic diversity for these crops. (Modified from *Crop Genetic Resources: An Economic Appraisal/EIB-2*, Economic Research Service/USDA, 2002).

A number of important transgene flow cases have been reported in centres of crop origin and diversity. Perhaps most widely known is the case of transgene introgression of maize in Oaxaca, Mexico (Quist & Chapela 2001; 2002). The substantial attention paid to reports on the status of transgenes in Mexican maize (Quist & Chapela 2001; NAFTA-CEC 2002; Alvarez Morales 2002; Quist & Chapela 2002; Cleveland et al. 2005; Ortiz-Garcia et al. 2005) has not translated into follow-up empirical studies on the evolutionary significance of transgenes in maize landrace populations. Given the occurrence of transgenic introgression events in Mexico, concerns have emerged over similar events taking place in other important crop plants, including rice and soya in China (Huang et al. 2003). The impending commercialization of GM rice has been met with considerable concern over gene flow to wild and weedy rice relatives (Lu & Snow 2005), and to non-transgenic commercial varieties. Given these events, and the uncertainties over the significance of transgenic hybridization, the introduction of transgenic crops in their centres of origin and diversification represents a broad concern with socio-economic and agricultural implications. Some of these impacts, particularly evolutionary implications, may be irreversible. For these reasons, transgenic introductions in centres of origin and diversification merit special consideration.

The issue of intellectual property rights (IPR) on crop cultivars adds another dimension to the issue of transgene flow. While IPRs are in conflict with the age-old practice of seed exchange amongst local farmers who use landraces, the introduction of identifiable transgenic technologies opens up the possibility that legal action could be taken against local farmers by the patent holders.²

While there has been greater attention paid to gene flow to wild relatives, there has been very little scientific study, descriptive or experimental, over the potential impacts of transgene

²See the case of Percy Schmeiser, a canola farmer from Canada (<http://www.percyschmeiser.com/>)

introgression in landraces. Clearly, establishment of transgenic hybrids in landrace populations is undesirable, given the high level of uncertainty as to their effects and incidence of gene movement. Policies that limit the planting of transgenic varieties in centres of origin have been widely recommended (NRC 2000; Eastham & Sweet 2002; Gepts & Papa 2003). Yet well-intentioned policies have been largely ineffective to date.

2.1.3 Crop to crop gene flow

Crop to crop gene flow, as previously mentioned, is a broad concern in areas of GM and non-GM cultivation or use of offspring's seeds. A number of 'gene spill' events of transgenics 'contaminating' non-transgenic crops, resulting from cross pollination (Friesen et al. 2003; Mellon & Rissler 2004), and sometimes seed mixing (Mellon & Rissler 2004) have been recorded. Transgenic introgression of conventional crops has its own share of biological, socio-economic, policy, and intellectual property concerns.

Of the biological considerations, the most significant is loss of non-transgenic genetic varieties, many of which are 'heirloom varieties' (landraces) of important crop diversity. It is important to note that this is also an issue with non-transgenic commercial hybrids, where the process of domestication of crops has led to genetic bottlenecks in virtually all crops analysed to date (Doebley 1992; Gepts 1993). This has the effect of limiting the genetic stocks available to farmers and breeders.

Socio-economically, many of the same concerns mentioned for landraces also exist with crop to crop transgene flow. A number of cases of inadvertent contamination of the food supply – particularly in the USA – with varieties not approved for human consumption have made recent headlines. Cases such as the Starlink corn contamination in 2000 (Kaufman 2000) and rice in the US with multiple transgenic varieties,³ are just a few examples of inevitable gene flow. Nations that do not accept (certain) GMO products have been forced to ban the import of grains or foods from these countries, causing a loss of markets for farmers and food distributors. Contamination events of organic crops can affect the premium value and genetic stocks of the crops for the affected farmers. Quite clearly, the unintended spread of transgenes has been a result of cultivation and seed distribution systems that were never designed for segregation of particular crop varieties. Human error and negligence of laws are also often to blame. Lastly, patent infringement lawsuits might be brought against farmers affected by transgene flow, as previously mentioned.

As a result of the many documented cases of transgene flow, robust monitoring programmes have been an important initiative for many countries, especially those with policies limiting GMOs in their food supply. Hence, tracking transgenes has not only biological but political implications.

3. Tracking transgenes

An essential initial component of understanding the ecological and environmental impact of transgene flow is first documenting the movement or presence of transgenes in a population, food shipment, or processed food item. This involves employing molecular methods to detect the synthetic transgenic DNA constructs, or target marker proteins introduced into the gene-modified commodity (Holst-Jensen et al. 2003; Nesvold et al. 2006).

³<http://www.guardian.co.uk/gmdebate/Story/0,,1884523,00.html> and http://www.aphis.usda.gov/publications/biotechnology/content/printable_version/ia_ge_rice.pdf

Successful monitoring and surveillance of transgenes in the environment or food shipment is reliant on a number of factors. First, one must be able to detect the transgenic sequences or proteins (see Chapter 33). Therefore *a priori* knowledge of the genes one is looking for is essential. Further, the gene sequence or protein one is targeting in the monitoring efforts must be intact and/or expressing. In addition, the sampling regime, limit of detection and reproducibility of results can further effect the outcome of any monitoring efforts, usually leading to false-negative results (Holst-Jensen et al. 2003). Hence, any sampling for GMOs is likely to underestimate the presence and/or frequency of GM DNA in a sampled population. Thus, not detecting a transgene in a sample population is no guarantee that the population is transgene free (Heinemann & Traavik 2004). Only with a careful multifaceted monitoring strategy can the accuracy and precision of our monitoring efforts be reasonably assured. Agencies dedicated to the detection of transgenes, such as the European Network of GMO Laboratories (ENGL) in the EU, have devised validated methods for the detection of transgenic DNA from approved GMOs in the European community. Thus, tracking transgenes is difficult, but not impossible.

4. Research needs, gaps in knowledge and uncertainties in gene flow assessments

In the first years following the commercialization of genetically modified organisms, the primary research focus has gone into developing detection systems and monitoring to account for unwanted GM DNA in foodstuffs and crops (as discussed). This has been motivated largely by policies of low or no GMO components in grain and foodstuffs in some countries, and has been a driving force in the science of GMO-related research. The salient question is the significance of gene flow when it occurs. Ecological studies of transgene flow have shed significant light on many of the unanticipated or unintended effects of transgenic biology, and have highlighted the need for robust science as the driving force behind risk assessments. Where ‘early warnings’ are identified (Harremoes et al. 2002), there is a need for careful consideration where lasting effects might otherwise be mitigated. The importance of context should not be lost on transgenic biology, where the behaviour of transgenes and their proteins might be very different within different biological (organismal) or ecological backgrounds.

While a much greater degree of risk science on transgene flow to date has focused on the direct ecological implications of specific transgenes, investigations into the ecological and evolutionary significance of transgene flow for genetic diversity in centres of origin are lacking. The case of transgenic maize in Mexico is one clear example of where such studies are urgently needed (Garcia et al. 1998; Quist & Chapela 2001; NAFTA-CEC 2002; Cleveland et al. 2005). As a result, many critical gaps in understanding remain on gene flow potential and barriers, including sexual compatibility, hybrid viability and fitness for many crop species.

Part of the difficulty in such studies is the lack of *a priori* predictive power given the likely variable behaviour of the transgene in new ecological and genetic backgrounds (Gepts & Papa 2003). Transgenic plants, like most commercial crop varieties, are designed for use within very specific environmental and cultural conditions of the agricultural field over one generation. They were never intended for new genomic or ecological backgrounds, or for use over subsequent generations that occur with gene flow. Much research has focused on the notion of fitness of a transgenic hybrid population to be substantially equivalent to transgenic crops within the intended agricultural setting. Conceptually, one must consider that the setting of the transgenic organism may grossly affect the effect or impact it may have within a particular milieu. For example, pest and competition pressures may be different depending on ecological setting, affecting fitness of the population much differently outside its intended agricultural context, such that equivalence of outcomes cannot be assumed. Further, hybridization into new genetic backgrounds may have a range of effects on the fitness of the recipient population. These responses may include a

metabolic cost decreasing its fitness, to a hybrid vigour increasing its costs. Outcomes may not be consistent across generations, growing ranges, climatic fluctuations, or stress pressures. A further consideration is the lack of understanding of the fate and stability of transgenes across generations (McCabe et al. 1999; Quist & Chapela 2001; Svitashv et al. 2002; Wilson et al. 2006) and post-translational silencing (Matzke et al. 2000), non-Mendelian inheritance, *pleiotropic* or *epistatic* effects (i.e. unintended changes in phenotype by the transgene introduction or interaction with other genes) that are important considerations for assessing gene establishment, expression, and hence fitness effects. Further, other levels of biological organization within the plant (transcriptome, proteome, metabolome; see Chapter 8) may also have direct impacts on fitness of gene flow. Another consideration is that the dominant currency of gene flow research as genes conferring traits assumes that all genes transferred will be protein-coding genes. This fails to consider the vast array of non-protein encoding DNA and RNA derivatives that are also implicated in the transfer of genetic information and the outcomes from one population to another (Mattick 2003).

Thus, the evolutionary implications of hybridization and introgression from crop to crop or crop to landrace/wild populations where it actually occurs are dependent on a number of factors, where the fitness effects cannot be predicted *a priori* to GM crop release, and may change over hybrid generations. Therefore, studies must be conducted on a case by case basis within any given context (country, environment, GMO, etc.) where relevant scientific questions can be addressed.

5. Practical considerations for policy and risk assessment on gene flow

5.1 Strategies for mitigating transgene flow

The knowledge gained from transgene flow studies has been useful in developing appropriate measures to limit gene flow from transgenic plants. A number of strategies have been outlined to document and minimize gene flow from transgenic sources.

Given the uncertainties over the ecological and evolutionary impacts of gene flow, the means to minimize potential gene flow are active areas of investigation. Most of these will involve temporal and spatial isolation of the transgenic crops from potential gene flow scenarios. Containment and confinement strategies span the range from the physical (Morris et al. 1994; Staniland et al. 2000) to the chemical (Schemthaler et al. 2003) to the molecular (Daniell 2002). No single strategy is failsafe, and overlapping approaches will be necessary to adequately ensure minimal transgene escape, yet must also be investigated for their own biosafety.

5.2 Context-specific considerations

The country, crop, and/or transgenic trait under consideration may be relevant to policy decisions on transgenic crops. For example, gene flow to landraces and wild relatives of maize may be an issue for a country such as Mexico, but not for Canada. Certain types of transgenic products may also trigger policy implications if they may impact sensitive non-target biodiversity. Foremost is a robust detection and monitoring system, whereby specific information on the marker DNA sequences, molecular characterizations and background knowledge on gene flow potential will all be important in any biosafety policy on transgenic crops. Lastly, beyond the possible ecological and economic implications of gene flow, the possible socio-economic costs of unintended gene flow must also be taken into account in any policy decision or risk assessment (Gepts & Papa 2003).

6. Conclusions

Emerging knowledge over the importance of the ecological, genetic and political backgrounds of GMO introductions is bringing new insights into the complexities surrounding the use of GMOs in agriculture. There is still much to be learned. Quite clearly, GMOs represent a new challenge in the management of agriculture where external costs and potential consequences must be duly measured along with and contrasted with any potential benefits. This is even more critical with the emerging use of crop plants to manufacture bioactive compounds, such as pharmaceuticals, that have an even greater risk magnitude. Given the scope, irreversibility and uncertainty surrounding the impacts of transgene flow, a critical analysis of the biological, ecological and social ramifications needs to be thoroughly examined to arrive at sound policy decisions. This requires asking the right questions – the relevant types of ‘what if’ risk questions—regarding the GMO under consideration within the right social, political and agroecological dimensions.

Literature cited

- (NAFTA-CEC), (2002). *Maize and Biodiversity: The effects of transgenic maize in Mexico: Key findings and recommendations*. Montreal, Commission for Environmental Cooperation: 35pp.
- Alvarez Morales, A. (2002). *Transgenes in maize landraces in Oaxaca: official report on the extent and implications*. The 7th International Symposium on the Biosafety of Genetically Modified Organisms, Beijing.
- Anderson, E. (1961). "The analysis of variation in cultivated plants with special reference to introgression." *Euphytica* 10: 79-86.
- Barton, J. and M. Dracup (2000). "Genetically modified crops and the Environment." *Journal of Agronomy* 92: 797-803.
- Bartsch, D., M. Lehnen, et al. (1999). "Impact of gene flow from cultivated beet on genetic diversity of wild sea beet populations." *Molecular Ecology* 8(10): 1733-1741.
- Bullock, J. (1999). "Using population matrix models to target GMO risk assessment." *Aspects of Applied Biology* 53: 205-212.
- Cleveland, D., D. Soleri, et al. (2005). "Detection (trans)gene flow to landraces in centers of crop origin: lessons from the case of maize in Mexico." *Environmental Biosafety Research* 4: 197-208.
- Crawley, M., R. Hails, et al. (1993). "Ecology of transgenic canola in natural habitats." *Nature* 363: 620-623.
- Daniell, H. (2002). "Molecular strategies for gene containment in transgenic crops." *Nature Biotechnology* 20(6): 581-586.
- Darmency, H. (1994). "The impact of hybrids between genetically modified crop plants and their related species: Introgression and weediness." *Molecular Ecology* 3(1): 37-40.
- Doebley, J. (1992). *Molecular systematics and crop evolution*. Molecular systematics of plants. S. PS, S. DE and J. Doyle. New York, Chapman Hall: 202-222.
- Eastham, K. and J. Sweet (2002). *Genetically modified organisms (GMOs): The significance of gene flow through pollen transfer*. Copenhagen, European Environment Agency: 75.
- Ellstrand, N. (2003). *Dangerous Liasons? When cultivated plants mate with their wild relatives*. Baltimore, MD, John Hopkins University Press.
- Ellstrand, N. C. (2001). "Crop transgenes in natural populations." *Abstracts of Papers American Chemical Society* 221(1-2): AGFD 37.
- Ellstrand, N. C. and C. A. Hoffman (1990). "Hybridization as an Avenue of Escape for Engineered Genes Strategies for Risk Reduction." *Bioscience* 40(6): 438-442.
- Ellstrand, N. C., H. C. Prentice, et al. (1999). *Gene flow and introgression from domesticated plants into their wild relatives*. 4139 El Camino Way, Palo Alto, CA, 94303-0139, USA, Annual Reviews.

- Ellstrand, N. C. and K. A. Schierenbeck (2000). "Hybridization as a stimulus for the evolution of invasiveness in plants?" *Proceedings of the National Academy of Sciences of the United States of America* 97(13): 7043-7050.
- Friesen, L., A. Nelson, et al. (2003). "Evidence of contamination of pedigreed canola (*Brassica Napus*) Seedlots in Western Canada with genetically engineered herbicide resistant traits." *Agronomy Journal* 95: 1342-1347.
- Garcia, M., J. Figueroa, et al. (1998). "Pollen control during transgenic hybrid maize development in Mexico." *Crop Science* 38(6): 1597-1602.
- Gepts, P. (1993). "The use of molecular and biochemical markers in crop evolution studies." *Evolutionary Biology* 27: 51-94.
- Gepts, P. and R. Papa (2003). "Possible effects of (trans)gene flow from crops on the genetic diversity from landraces and wild relatives." *Environmental Safety Research* 2: 89-103.
- Gueritain, G., M. Sester, et al. (2002). "Fitness components of progeny of hybrids between transgenic oilseed rape (*Brassica napus*) and wild radish (*Raphanus raphanistrum*)." *Molecular Ecology* 11: 1419-1426.
- Haygood, R., A. R. Ives, et al. (2003). "Consequences of recurrent gene flow from crops to wild relatives." *Proceedings of the Royal Society of London – Series B: Biological Sciences* 270(1527): 1879-1886.
- Hall, L., K. Topinka, et al. (2000). "Pollen flow between herbicide-resistant *Brassica napus* is the cause of multiple-resistant *B. napus* volunteers." *Weed Science* 48(6): 688-694.
- Harremoes, P., D. Gee, et al., Eds. (2002). *Late lessons from early warnings: the precautionary principle 1896-2000*. Luxembourg, European Environment Agency.
- Hauser, T. P., R. B. Jorgensen, et al. (1998). "Fitness of backcross and F2 hybrids between weedy *Brassica rapa* and oilseed rape (*B. napus*)." *Heredity* 81(4): 436-443.
- Heinemann, J. A. and T. Traavik (2004). "Problems in monitoring horizontal gene transfer in field trials of transgenic plants." *Nature Biotechnology* 22(9): 1105-1109.
- Holst-Jensen, A., S. B. Ronning, et al. (2003). "PCR technology for screening and quantification of genetically modified organisms (GMOs)." *Annals of Bioanalytical Chemistry* 375: 985-993.
- Huang, J., S. Rozell, et al. (2003). "Plant biotechnology in China." *Science* 295: 674-676.
- Jenczewski, E., J. Ronfort, et al. (2003). "Crop-to-wild gene flow, introgression and possible fitness effects of transgenes." *Environmental Biosafety Research* 2: 9-24.
- Kaufman, M. (2000). *Biotech critics cite unapproved corn shells*. Washington Post. Washington, D.C.
- Lavigne, C., E. K. Klein, et al. (1998). "A pollen-dispersal experiment with transgenic oilseed rape. Estimation of the average pollen dispersal of an individual plant within a field." *Theoretical and Applied Genetics* 96(6-7): 886-896.
- Lenormand, T. (2002). "Gene flow and the limits to natural selection." *Trends in Ecology & Evolution* 17: 183-189.
- Linder, C. and J. Schmitt (1995). "Potential persistence of escaped transgenes: performance of transgenic, oil-modified *Brassica* seeds and seedlings." *Ecological Applications* 5: 1056-1068.
- Linder, C. R. (1998). "Potential persistence of transgenes: Seed performance of transgenic canola and wild X canola hybrids." *Ecological Applications* 8(4): 1180-1195.
- Linder, C. R., I. Taha, et al. (1998). "Long-term introgression of crop genes into wild sunflower populations." *Theoretical and Applied Genetics* 96(3-4): 339-347.
- Lu, B.-R. and A. Snow (2005). "Gene flow from genetically modified rice and its environmental consequences." *Bioscience* 55(8): 669-678.
- Mattick, J. S. (2003). "Challenging the dogma: the hidden layer of non-protein-coding RNAs in complex organisms." *Bioessays* 25(10): 930-939.

- Matzke, M. A., M. F. Mette, et al. (2000). "Transgene silencing by the host genome defense: Implications for the evolution of epigenetic control mechanisms in plants and vertebrates." *Plant Molecular Biology* 43(2-3): 401-415.
- McCabe, M. S., U. B. Mohapatra, et al. (1999). "Integration, expression and inheritance of two linked T-DNA marker genes in transgenic lettuce." *Molecular Breeding* 5(4): 329-344.
- Mellon, M. and J. Rissler (2004). *Gone to Seed: Transgenic Contaminants in the traditional seed supply*. Cambridge, MA, Union of Concerned Scientists.
- Messeguer, J. (2003). "Gene flow assessment in transgenic plants." *Plant Cell, Tissue and Organ Culture* 73: 201-212.
- Mikkelsen, T., J. Jensen, et al. (1996). "Inheritance of oilseed rape (*Brassica napus*) RAPD markers in a backcross progeny with *Brassica campestris*." *Theoretical & Applied Genetics* 92: 492-497.
- Morris, W., P. Kareiva, et al. (1994). "Do barren zones and pollen traps reduce gene escape from transgenic crops?" *Ecological Applications* 4(1): 157-165.
- Nesvold, H., A. Kristoffersen, et al. (2006). "Design of a DNA chip for detection of unknown genetically modified organisms (GMOs)." *Bioinformatics* 21(9): 1917-1926.
- NRC, Ed. (2000). *Environmental effects of transgenic plants: the scope and adequacy of regulation*. Washington, D.C., National Academies Press.
- Ortiz-Garcia, S., E. Ezcurra, et al. (2005). "Absence of detectable transgenes in local landraces of maize in Oaxaca, Mexico (2003-2004)." *Proceedings of the National Academy of Sciences of the United States of America* 102(35): 12338-12343.
- Parker, I. M. and P. Kareiva (1996). "Assessing the risks of invasion for genetically engineered plants: Acceptable evidence and reasonable doubt." *Biological Conservation* 78(1-2): 193-203.
- Pessel, D., J. Lecomte, et al. (2001). "Persistence of oilseed rape (*Brassica napus* L.) outside of cultivated fields." *Theoretical & Applied Genetics* 102: 841-846.
- Pilson, D. and H. R. Prendeville (2004). "Ecological effects of transgenic crops and the escape of transgenes into wild populations." *Annual Review of Ecology Evolution and Systematics* 35: 149-174.
- Quist, D. and I. H. Chapela (2001). "Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico." *Nature (London)* 414(6863): 541-543.
- Quist, D. and I. H. Chapela (2002). "Maize transgene results in Mexico are artefacts – Reply." *Nature* 416(6881): 602.
- Reichman, J., L. Watrud, et al. (2006). "Establishment of transgenic herbicide-resistant creeping bentgrass (*Agrostis stolonifera* L.) in nonagronomic habitats." *Molecular Ecology* 15: 4243-4255.
- Reiseberg, L. and J. Wendel (1993). *Introgression and its evolutionary consequence in plants. Hybrid Zones and the Evolutionary process*. R. Harrison. New York, Oxford University Press: 70-109.
- Schemthaner, J., S. Fabijanski, et al. (2003). "Control of seed germination in transgenic plants based on the segregation of a two-component genetic system." *Proceeding of the National Academy of Science, USA* 100: 6855-6859.
- Simard, M., A. Legere, et al. (2002). "The frequency and persistence of canola (*Brassica napus*) volunteers in Quebec cropping systems." *Weed Technology* 16: 433-439.
- Singh, S., A. Molina, et al. (1995). "Potential of wild common bean for seed yield improvement of cultivars in the tropics." *Canadian Journal of Botany* 75: 807-813.
- Snow, A. A., D. A. Andow, et al. (2004). *Genetically engineered organisms and the environment: Current status and recommendations*. Washington, D.C., Ecological Society of America: 38.
- Snow, A. A. and P. M. Palma (1997). "Commercialization of transgenic plants: Potential ecological risks." *Bioscience* 47(2): 86-96.

- Snow, A. A., D. Pilson, et al. (2003). "A Bt transgene reduces herbivory and enhances fecundity in wild sunflowers." *Ecological Applications* 13(2): 279-286.
- Snow, A. A., K. L. Uthus, et al. (2001). "Fitness of hybrids between weedy and cultivated radish: Implications for weed evolution." *Ecological Applications* 11(3): 934-943.
- Spencer, L. J. and A. A. Snow (2001). "Fecundity of transgenic wild-crop hybrids of *Cucurbita pepo* (Cucurbitaceae): Implications for crop-to-wild gene flow." *Heredity* 86(6): 694-702.
- Staniland, B., P. McVetty, et al. (2000). "Effectiveness of border areas in confining the spread of transgenic *Brassica napus* pollen." *Canadian Journal of Plant Science* 80: 107-114.
- Svitashev, S. K., W. P. Pawlowski, et al. (2002). "Complex transgene locus structures implicate multiple mechanisms for plant transgene rearrangement." *The Plant journal : for cell and molecular biology* 32(4): 433-445.
- Wilson, A., J. Latham, et al. (2006). "Transformation-induced mutations in transgenic plants: Analysis and biosafety implications." *Biotechnology and Genetic Engineering Reviews* 23: 209-234.