

TRANSGENE INTROGRESSION FROM GENETICALLY MODIFIED CROPS TO THEIR WILD RELATIVES

C. Neal Stewart Jr^{*}, Matthew D. Halfhill^{*} and Suzanne I. Warwick[‡]

Transgenes engineered into annual crops could be unintentionally introduced into the genomes of their free-living wild relatives. The fear is that these transgenes might persist in the environment and have negative ecological consequences. Are some crops or transgenic traits of more concern than others? Are there natural genetic barriers to minimize gene escape? Can the genetic transformation process be exploited to produce new barriers to gene flow? Questions abound, but luckily so do answers.

GENETIC MODIFICATION

INTROGRESSION

The permanent incorporation of genes from one set of differentiated populations (species, subspecies, races and so on) into another.

LANDRACES

A crop cultivar that evolved with and has been genetically improved by traditional agriculturalists, but has not been directly influenced by modern breeding practices.

Transgene **INTROGRESSION** from genetically modified (GM) crops into wild relatives is an issue that has rarely been out of the news since the publication, in *Nature*, in late 2001, of a paper entitled 'Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico'¹. After much controversy, *Nature* retracted the paper², because introgression³ *per se* was not shown⁴. Kernels on separate cobs of a Mexican **LANDRACE** showed positive, but weak, PCR signals, which pointed to the presence of a transgene that had been genetically engineered into maize crops growing in the same region. However, these weak signals indicated that only a few kernels on any particular ear of maize might be transgenic⁴. **F₁ HYBRIDIZATION** between the GM crops and the landraces could have occurred, but not introgression of the transgene, which would have required repeated **BACKCROSSES** and the stabilization of the transgene in the new host genome. If introgression had occurred, most of the kernels on a given ear would be transgenic. No published data have ever shown that transgenic DNA has been unintentionally introgressed into maize landraces or into any unintended maize genome.

This high-profile case illustrates the misunderstandings that can arise through the confusion of hybridization and **GENE FLOW** with introgression. Introgression, although based on hybridization, is not synonymous

with hybridization and gene flow. The distinction is important because the risk that is associated with the introgression of transgenes from transgenic crops into related weeds and free-living wild relatives is the focus of much concern: the worry being that transgenes will persist in the environment in new unintended hosts and have negative ecological consequences. As well as emphasizing the importance of this distinction, we argue that it is too simplistic to say that transgene introgression in itself poses a risk without considering the transgenic trait of interest, the crop and the cropping system.

Transgene dispersal from GM crops to wild relatives is often seen simply as pollen flow from the crop to the relative. The process of introgression is not this simple and actually occurs in many steps that involve several hybrid generations (**F₁**, **F₂**, **BC₁**, **BC₂** and so on), all of which can exchange genes and coexist simultaneously for many years (**FIG. 1**). Transgenic crop varieties might also form volunteer populations; (that is, germinate from the seedbank to form unwanted 'weedy' plants in the crops of subsequent years). These volunteer populations might be important reservoirs from which a transgene could be passed into the genome of a wild relative. Therefore, introgression of a crop transgene is a dynamic process that might take many years and generations before the transgene is fixed in the genetic background

^{*}Department of Plant Sciences, University of Tennessee, Knoxville, Tennessee 37996, USA.
[‡]Agriculture and Agri-Food Canada, Eastern Cereal and Oilseeds Research Centre, K.W. Neatby Bldg., C.E.F., Ottawa, Ontario K1A 0C6, Canada.
Correspondence to C.N.S.
e-mail: nealstewart@utk.edu
doi:10.1038/nrg1179

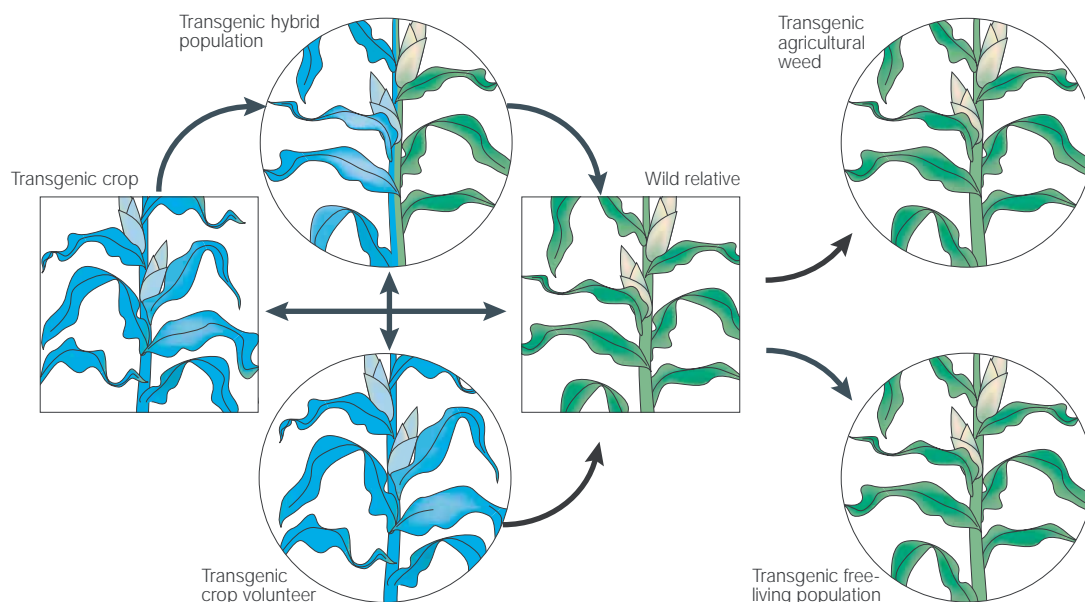


Figure 1 | **Transgene flow and potential transgene reservoirs.** The boxes represent initial populations and arrows represent potential gene-flow pathways. The circles represent potential transgenic populations after the introduction of transgenes and introgression.

of a wild relative. However, if selection is strong and/or population size is small, fixation of an introgressed gene could occur rapidly.

The issue of transgene introgression from crops into non-crop relatives is fraught with misinformation, fear and confusion. 'Frankenweeds', 'superweeds' and even 'transgenic weeds' are labels that illustrate the hype that surrounds this subject. Here, we consider questions of transgene introgression. Rather than seeking to address whether introgression can occur, we examine whether it is likely to occur with transgenes in the main annual crop species. It is timely to address these issues because, although the large-scale commercial release of transgenic crops continues, a large section of the public still believes that the environmental risks associated with gene flow have not been adequately addressed. First, we look at the lessons that have been learned from natural introgression, crop breeding and evidence of crop-to-wild introgression. Second, given this information, we attempt to predict which transgenes might be introgressed into the free-living relatives of transgenic plants; fortunately, we are not stuck in a natural or technological rut and technologies that can limit gene flow are discussed. Third, and finally, we pinpoint the research and the methods that are required to address regulatory and scientific needs with regards to transgene introgression.

Natural introgression

Nature is full of discrete species and although there are extensive **HYBRID ZONES**, they are the exception rather than the rule⁵. Nonetheless, the study of introgression in plants has received much attention because it is considered by many botanists to be important in plant evolution^{3,6–12}. The potential consequences of natural introgression include increased genetic diversity, the

transfer of adaptations, the origin of adaptations, the origin of ecotypes or species, the breakdown or reinforcement of isolating barriers and the promotion of colonization and dispersal^{3,9–11}.

Frequency of introgression. There is evidence that tens of thousands (the actual figure is probably higher) of plant taxa can hybridize, but the number of confirmed cases of introgression is much smaller. Rieseberg and Wendel³ listed 165 proposed cases of introgression, 65 of which were deemed to be sufficiently documented. These cases are from many different families and include plants with diverse growth forms, pollen-dispersal strategies and mating systems³. This list is far from complete or unbiased. In particular, there is a tendency for more recent introgression events and those that have occurred between distantly related taxa to be preferentially detected. This bias reflects the decreased likelihood of detecting introgression between closely related species because of the lack of diagnostic molecular and morphological markers. Also, there is a decreased likelihood of detecting less recent introgression events because there has been more time for mutation and/or random genetic loss to disguise genes that have been transferred from another genome. Therefore, the confirmed cases of introgression represent the minimum number of actual introgression events³.

Approximately two-thirds of the documented cases are of introgression between differentiated subspecies rather than species. The bias against detecting introgression between closely related taxa indicates that even this high proportion might be an underestimate. In terms of assessing the risks of introgression from GM crops, this is good news: introgression between species is of more concern than introgression between subspecies because

F1 HYBRIDIZATION

The initial cross between parent plants of different varieties, subspecies, species or genera.

BACKCROSSES

The mating of an individual with its parent, or with an individual of the same genotype as its parent, to follow the inheritance of alleles and phenotypes.

GENE FLOW

The dispersal of genes, in both gametes and zygotes, in and between breeding populations.

BC₁ AND BC₂ HYBRID

The offspring of a cross between a hybrid and one of the recurrent parent species or varieties. The subscript number represents the number of generations that have been crossed in this fashion.

HYBRID ZONES

(Hybrid swarms). Areas in which hybrid plants backcross to the parents and cross with themselves, so there is a continuous intergradation of forms in the population.

ISOZYME

Different forms of the same enzyme (synonymous with allozymes), which were used as some of the first biochemically-based genetic markers.

ALLOPATRIC

Occurring in geographically separate areas.

LINKAGE DISEQUILIBRIUM

(LD). A statistical measure of the non-independence of alleles. Departure from the predicted frequencies of multiple locus gamete types, assuming that all alleles are randomly associated.

CLINE

A variational trend in space that is found in a population, or a series of populations, of a species.

GENETIC LINKAGE MAP

A linear map of the relative positions of genes along a chromosome. Distances are established by linkage analysis, which determines the frequency at which two gene loci become separated during chromosomal recombination.

of the possibility of creating more aggressive transgenic weedy or invasive species.

Proposed cases of plant introgression have often lacked the necessary information to confirm, deny or estimate its extent. Introgression can be confused with evolutionary convergence and lineage sorting. Also, low levels are difficult to detect without several suitable interspecific genetic markers. Molecular markers that are based on DNA polymorphisms have provided a much-needed tool to study introgression.

Molecular markers for studying introgression. The use of molecular markers has greatly increased our ability to detect and quantify interspecific gene exchange (reviewed in REFS 3, 12, 13). They are particularly useful for analysing ambiguous cases of natural introgression. Over the years, the most conclusive evidence for introgression has come from molecular data: initially from ISOZYME work and more recently from DNA data^{3, 12, 13}.

Molecular markers are excellent for tracking introgression as they tend to be neutral and effectively unlimited in number. So, sufficient independent taxon-specific molecular markers can be obtained to detect even extremely low levels of introgression. It is also possible to monitor both nuclear and cytoplasmic gene flow, which might reflect different histories of gene exchange (BOX 1). Studies of the molecular phylogenies of several crops and their wild relatives have also been instrumental in detecting both recent and ancient introgression events in plant species.

Hybrid zones and genetic barriers. Studies of natural hybrid zones have indicated that genetic isolation should be viewed as a property of individual genes or chromosomal segments, not as a characteristic of entire genomes¹⁴. The units of selection in hybrid zones are chromosomal blocks^{15, 16}, which might be positively selected for, neutral or selected against (FIG. 2). A natural hybrid zone might be semi-permeable (that is, the recipient genome has regions or 'hot spots' in which introgression is more likely). For example, in studies of interspecific gene flow from *Helianthus petiolaris* to *Helianthus annuus*, the regions of the genome of *H. annuus* that seemed to accept foreign alleles or chromosomal segments of *H. petiolaris* had the same gene order or chromosomal arrangement in both species¹⁶ (in other words, the chromosomal areas in which the gene order differed did not accept foreign genes). So, chromosomal rearrangements seem to pose an important barrier to introgression. Even when the genome of a specific taxon is able to accept foreign DNA, the fate of a specific foreign chromosomal segment can vary: it can be transmitted relatively intact, partially transmitted or excluded completely, depending on the genes that it contains. For example, if a transgene is in strong LINKAGE DISEQUILIBRIUM (LD) with an allele that is selected against when transferred to the wild relative, then introgression of the transgene is unlikely to occur.

In summary, because natural hybrid zones contain a range of genotypes that are the result of many generations of recombination and natural selection, and all components of the barriers to interspecific gene flow or introgression are represented, they can be useful models to study the movement of transgenes between species. Experimental approaches that are used in natural introgression are directly applicable for tracking transgenes, including measurement of the CLINE width of dispersed alleles or molecular markers, assessment of patterns of LD and the use of mapped molecular markers (that is, GENETIC LINKAGE MAPS) to map specific traits that contribute to genetic isolation. In natural hybrid zones, the introgression of alleles that are negatively selected in the habitat or genetic background of the 'other' taxon will be retarded, whereas neutral or positively selected alleles will introgress at higher frequencies and across greater distances^{14, 17}. Similar effects are predicted for the establishment and spread of neutral, negatively selected and positively selected transgenes in wild populations.

Targeted introgression in plant breeding

The development of crop varieties for agricultural purposes has provided some of the most detailed studies of the mechanics of introgression. The introgression of beneficial exotic genetic material into crop varieties extends the potential variability of the crop, even allowing for the introduction of traits that were originally absent from the genome^{18–20}. Many ELITE CROP VARIETIES might be improved by the incorporation of alleles, and occasionally new genes, from exotic sources. The introgression of transgenes from crops to wild relatives might be viewed as a similar process. Introgression breeding or directed introgression can be a powerful model to examine this

Box 1 | Introgression in Louisiana irises

One of the best characterized examples of natural introgression is between two perennial iris species — *Iris fulva* (2n=42, shaded understory habitat along the banks of bayous) and *Iris hexagona* (2n=44, open freshwater marshes and swamps) — with many plants of intermediate phenotype observed in areas that have been disturbed by man⁶. A range of species-specific molecular markers (rDNA, allozymes and random amplified polymorphic DNAs) have confirmed localized introgression^{95, 96}. Swamp habitats contain a mixture of hybrids and a few *I. hexagona* plants; whereas in marsh habitats, although a range of hybrid types are found, the frequency of *I. fulva* markers is higher. Maternally inherited chloroplast DNA (cpDNA) markers have provided evidence for asymmetrical introgression, with most hybrids having *I. hexagona* as the female parent and *I. fulva* as the male parent. Bidirectional introgression was confirmed by the presence of marker genes for *I. fulva* in an ALLOPATRIC population of *I. hexagona* that was 10 km from the nearest *I. fulva* population, and by the presence of *I. hexagona* markers in *I. fulva* plants that were 25 km from the nearest *I. hexagona*⁹⁷. Hybridization and introgression also led to the formation of *Iris nelsonii*, which is a fixed derivative of the hybridization of *I. fulva*, *I. hexagona* and *Iris brevicaulis*⁹⁸.

Various studies of relative fitness in response to shade and salinity tolerance, and to interspecific competitive ability have been done on the Louisiana iris hybrids⁹⁷. *I. fulva*-like plants have intermediate or higher fitness than the parent species (*I. fulva* and *I. hexagona*), whereas *I. hexagona*-like plants have intermediate or equivalent relative fitness⁹⁹. A range of fitness values has been observed in *I. fulva* and *I. brevicaulis* hybrids¹⁰⁰, with some equivalent to and others lower in fitness than the parent species.

The iris introgression studies clearly show the persistence and spread of genes as a result of three main introgressive patterns: local geographical formation of hybrid swarms, gene flow beyond the range of the original hybridization zone and formation of a new stabilized taxon.

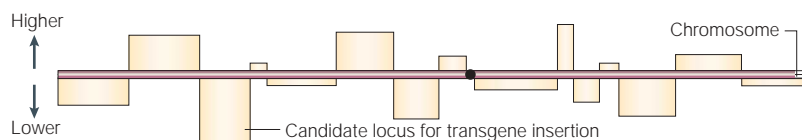


Figure 2 | Chromosomal blocks are the unit of selection in hybrid and introgression zones.

Certain chromosomal blocks might contain genes that are positively selected for and therefore have a higher likelihood of being transferred into the genome of related species and of conferring higher fitness to progeny. Negatively selected blocks might also occur, which have a negative effect on the fitness of hybrid plants and are less likely to be introgressed into the genome of related species. It is therefore desirable to engineer transgenes in loci that are located in regions of the crop genome that confer lower FITNESS when transferred to a wild relative.

process in an artificial setting and to predict how it might function when transgenes are moved from crops to wild relatives.

Crop lines have been bred for specific domestication traits^{21,22} (such as lack of seed dormancy or SEED SHATTERING), whereas EXOTIC CROP VARIETIES retain many naturally selected wild or weedy traits^{23,24}. The genetic basis of most exotic traits is not well-characterized, however anecdotal evidence indicates that the success of exotic × elite crosses is often limited because the beneficial exotic gene(s) that are under selection are linked to unselected or deselected alleles that reduce agronomic quality. As the mechanism for decreasing LD is recombination, LD between the beneficial exotic gene and unselected loci is expected to be highest in clonal species (in which the whole genome is essentially linked), intermediate in species that are predominantly selfing and lowest in predominantly outcrossing species^{25–27}.

The artificial selection of genes with the aim of introgressing them from exotic sources into elite cultivars can be viewed as a similar process to the natural selection of transgenes that are unintentionally transferred from crops into wild populations. However, there are some important differences between purposely introgressing genes into a crop and the unintentional movement of genes in the opposite direction (that is, the introgression of crop genes to wild species). When crop breeders introgress exotic alleles they plan on 5–10 backcrosses to overcome LD. However, the number of generations that are necessary to overcome LD when alleles move from crops to wild plants varies on the basis of natural selection pressure. Another difference is the strong selection that is applied for crop end-points by breeders, versus the unpredictable (generally weaker) selection that is provided by nature. The expected lower fitness of domestication genes in a wild setting might counteract any positive fitness effects of genes that are in LD with them, if they are transferred from a crop to a wild relative. Domestication genes are expected to cause an even bigger reduction in fitness if they are transferred into a weedy compared with a non-weedy wild relative because they might decrease the potential for weediness and lead to maladaptation. So, introgression from a crop to its wild relative is generally regarded as more difficult than from the wild relative to the crop. Although

we have more information on wild-to-crop introgression under controlled conditions, studies of natural introgression (crop to wild, wild to crop and wild to wild) might be more applicable.

In summary, targeted introgression in plant breeding has indicated that prolonged strong selection is often required to effectively introgress a specific gene and such introgression is often associated with a suite of linked alleles from the exotic parent. We should expect a similar degree of linkage when transgenes flow from crops to wild relatives. Plant varieties that are developed from WIDE CROSSES frequently fail to develop the agronomic characteristics of the elite parent; this is often attributed to the LD associated with the introgressed trait. In terms of the introgression of transgenes from GM crops to wild plant populations, the linkage of domestication alleles might impose an important barrier to prevent wild relatives from stably acquiring new transgenic phenotypes from crops.

Introgression from crops to wild relatives

Although examples of natural introgression and targeted introgression in plant breeding are informative, the main concern about transgene introgression involves crops and their free-living wild relatives (TABLE 1). Of particular concern is the unidirectional introgression of crop transgenes into non-crop plants. The wild relatives can be of the same or a related species. Wild relatives can range from noxious weeds (unwanted plants that are associated with disturbed habitats and compete with crops) to non-weedy species that occupy more natural habitats. Hypothetically, a fitness-enhancing transgene could increase the potential weediness of the wild relative, and this is considered to be of most concern in wild relatives that are already weedy. The prospects of altering the ecology of the recipient wild relative must be assessed on a case-by-case basis.

Researchers have approached the assessment of the possibility of introgression between crops and wild relatives in various ways. Prompted by the possibility of transgene introgression, Haygood *et al.*²⁸ recently used mathematical modelling to argue that crop genes and transgenes could have profound negative effects on wild relatives. However, their predictions, including the pervasive presence of crop alleles in wild plants, are not substantiated by empirical findings. There seems to be little evidence in nature for the MIGRATIONAL MELTDOWN and shrinkage of wild populations from crop gene introgression that their modelling predicts²⁸. Ellstrand *et al.*²⁹ assessed the broad possibility of introgression of genes from 13 important crops to their wild relatives. They found that 12 of the 13 crops with the greatest worldwide acreage might hybridize with wild relatives, seven of which have putatively introgressed²⁹. Also, five other crops might possibly be subject to introgression.

In contrast to previous approaches, we focus on whether introgression to wild relatives will occur when crops are genetically modified. We are specifically interested in transgenes, not endogenous crop genes, and in empirical data rather than models. Using this approach, we consider transgenic crops that are grown in regions

ELITE CROP VARIETIES

Agronomically desirable crop varieties that are widely used, adapted to local environments, perform well under intensive agricultural practices and are typically the product of intensive breeding.

SEED SHATTERING

Seeds dispersing from their fruits before harvest.

EXOTIC CROP VARIETIES

A variety that is from outside a breeding region or has traits that are uncommon to the prevalent crop variety.

FITNESS

The potential evolutionary success of a genotype, which is defined as the reproductive success or the proportion of genes that an individual leaves in the gene pool of a population. The individuals with the greatest fitness leave the largest numbers of offspring.

WIDE CROSSES

Hybridization between differentiated taxa.

MIGRATIONAL MELTDOWN

The theoretical state of gene flow that leads to the fixation of a 'bad' gene and the subsequent reduction of population size.

Table 1 | Crops from which transgenes might introgress into wild relatives

| Crop | Main species | Progenitor species | Genomic structure, chromosome number (2n) | Transgenic varieties | Main wild relatives (2n) (distribution) | References* |
|------------|---|---|--|--|--|------------------------------|
| Sorghum | <i>Sorghum bicolor</i> | <i>S. bicolor</i> | Two subgenomes of five chromosomes (2n=10) | None | <i>S. bicolor</i> (2n=20) (Old and New World) <i>Sorghum halepense</i> (2n=40) (Old and New World) <i>Sorghum propinquum</i> <i>Sorghum alnum</i> | 106,107 65,66 66 66 |
| Sunflower | <i>Helianthus annuus</i> | <i>H. annuus</i> | (2n=34) | Herbicide and insect tolerance in development | <i>H. annuus</i> (2n=34) (New World) <i>Helianthus petiolaris</i> (2n=34) (New World) | 62,63 16,64 |
| Canola | <i>Brassica napus</i> | <i>Brassica rapa</i> and <i>Brassica oleracea</i> | AACC (2n=38) | Herbicide tolerance commercially released, insect and disease stress tolerance | <i>B. rapa</i> (AA, 2n=20) (widespread in most canola growing areas) | 57 [‡] |
| | <i>B. rapa</i> | <i>B. rapa</i> | AA (2n=20) | Commercial release withdrawn | <i>B. rapa</i> (AA, 2n=20) (widespread in most canola growing areas) | – |
| Wheat | <i>Triticum aestivum</i> | Wild <i>Triticum</i> and <i>Aegilops</i> species | AABBDD (2n=42) | Herbicide tolerance developed, not yet commercially released | <i>Aegilops cylindrica</i> (DD, 2n=28) (North America) | 54, 55 |
| Sugar beet | <i>B. vulgaris</i> ssp. <i>vulgaris</i> | <i>B. vulgaris</i> ssp. <i>maritima</i> | (2n=18/27) | Herbicide and disease (virus) tolerance commercially released | <i>B. vulgaris</i> ssp. <i>maritima</i> (2n=18) (Old World) | 52,53,108,109 |
| Alfalfa | <i>Medicago sativa</i> ssp. <i>sativa</i> | <i>M. sativa</i> | (2n=32) | Herbicide and stress tolerance developed but not commercially released | <i>M. sativa</i> ssp. <i>sativa</i> (ssp. <i>caerulea</i>) (2n=16) (Old and New World) <i>M. sativa</i> ssp. <i>falcata</i> (2n=16/32) (Old and New World) | 50,51 |

* Examples of molecular evidence for crop-to-wild introgression. † S.I.W., unpublished data.

away from their wild relatives — for example, soybean in the United States — not to be risky and therefore place them in a lower risk category than Ellstrand *et al.*²⁹. We have assessed the molecular evidence for crop-to-wild introgression in these crops, together with others that are potential targets for genetic modification. On the basis of this assessment, we divide these crops into four risk categories for the introgression of transgenes: very low risk, low risk, moderate risk and high risk. Other authors^{30,31} have used a similar risk-assessment categorization approach, as well as a detailed decision-tree-based risk-assessment categorization methodology³².

Very low risk crops. There is no molecular evidence of crop-to-wild introgression in soybean (*Glycine max*), barley (*Hordeum vulgare*), finger millet (*Eleusine coracana*), pearl millet (*Pennisetum glaucum*), common bean (*Phaseolus vulgaris*), peanut (*Arachis hypogaea*) and potato (*Solanum tuberosum*). The lack of evidence is notable in some cases, given the evidence of genetic compatibility with wild relatives. In soybean, for example, the crop *G. max* and its wild relative *Glycine soja* are highly similar at the genomic level³³, and several studies^{34,35} have shown introgression of DNA from *G. soja* into *G. max*. So far, there are no reports of crop-to-wild introgression in soybean, although it is unclear how many attempts there have been to assess this. Similarly, in peanut, it is clear that genes can be introgressed from wild to crop species^{36,37}. In the common bean, a phaseolin protein marker has been

found in what seem to be wild × crop hybrid swarms in the Andes Mountains³⁸. However, molecular evidence shows that Andean and Mesoamerican bean landraces in Chile are genetically distinct from one another and there is no evidence of introgression between them³⁹. This genetic differentiation indicates that introgression is limited to the geographic centre of diversity.

We suggest that the transgenic forms of these crops are at a very low risk of introgressing their transgenes into wild populations. Given this fact, we believe that most transgenes could be safely engineered into these very low risk crops.

Low risk crops. Some crops have been shown to introgress genes into wild relatives at low levels. Although such rare events could be evolutionarily important, in these cases the biology and distribution of wild relatives can alter and often moderate any ecological risks. So, engineered crops such as corn, rice and cotton do not seem to pose large risks for agricultural or ecological stability. Therefore, transgenes could be engineered into these species, although as a precautionary measure the release of transgenic lines should be restricted to areas in which the wild relatives do not occur.

Corn (*Zea mays* ssp. *mays*) can hybridize with inter-specific wild relatives, that are known collectively as teosinte^{40,41}, from which it was domesticated 9,000 years ago⁴². However, gene flow seems to be primarily unidirectional from teosinte to corn⁴³, with insignificant levels of introgression from corn to teosinte^{40–41}.

Molecular evidence for rice (*Oryza sativa*) introgression is limited compared with morphological data sets (for example, see REF. 44 and references therein). However, studies of natural weedy Asian rice (*O. sativa*) populations showed isozyme introgression into local populations of wild *Oryza rufipogon* in Thailand^{45,46}. Controlled F₁ field hybridization studies^{47,48} between crop rice and *O. rufipogon* indicate that hybridization rates are low (1–2%), partly because competing crop pollen on the flowers of the wild species acts as a barrier to hybridization⁴⁷. However, some gene flow between the crop and the wild species, as well as gene flow among other wild rice species, can occur⁴⁸. Nonetheless, analysis of miniature inverted-repeat transposable elements (MITEs) shows conserved patterns among rice species and ECOTYPES, which indicates that there is little introgression over an evolutionary timeframe⁴⁹.

Ellstrand *et al.*²⁹ cited several studies that show molecular evidence of introgression at low frequencies for both of the cotton species: *Gossypium hirsutum* (95% of the worldwide cotton crop) and *Gossypium barbadense*. However, introgression of crop transgenes into wild *G. hirsutum* is unlikely, as wild *G. hirsutum* is distributed in the New World tropics and not in prime cotton-growing areas.

Moderate risk crops. There is ample evidence for introgression between crops and wild relatives in alfalfa, sugar beet, wheat, canola and sunflower. The wild relatives of these species can form extensive free-living populations and, in some cases, are agricultural weeds. The potential ecological effects of the transgene must be considered on a case-by-case basis for these species (BOX 2). The introduction of herbicide-tolerance transgenes could complicate weed-management practices, although the environmental effects would be limited to the agroecosystem in which the herbicide is applied. Transgenes that confer stress tolerance or resistance to

insects or diseases could also cause ecological or agronomic problems. However, further work is needed to definitively assess the risks that are associated with introducing such transgenes into these crops.

Alfalfa (*Medicago sativa* ssp. *sativa*) introgresses with wild *M. sativa*^{50,51}, which is found along roadsides and on wasteland. Genes from sugar beet (*Beta vulgaris* ssp. *vulgaris*), which is biennial, can introgress into wild beet (the same species) and sea beet (*Beta vulgaris* ssp. *maritima*) at low frequencies^{52,53}. Only a small proportion of the agricultural populations of these two crops will ever be genetically modified for niche markets in which specific traits that are introduced through transgenes would add value. However, in both cases, if genes for herbicide tolerance were engineered into crop populations they would probably introgress into their wild relatives and produce herbicide-tolerant weeds.

Bread wheat (*Triticum aestivum*) and durum wheat (*Triticum turgidum* ssp. *turgidum*) crops can hybridize with wild *T. turgidum* and *Aegilops* species. Of most concern is the risk of gene flow from bread wheat into jointed goatgrass (*Aegilops cylindrica*), which is a serious weed of cereal production⁴⁷. This possibility has slowed the commercialization of glyphosate-tolerant wheat. Although F₁ hybrids between wheat and jointed goatgrass are mostly sterile, BC₁ and BC₂ hybrids can be produced with increasing fertility^{54,55}. Field surveys in Oregon⁵⁶ showed a 1% backcross seed-production rate, but field-level introgression has yet to be confirmed.

Canola (*Brassica napus*) can hybridize with many species, but the best data in support of introgression are from studies with field mustard (*Brassica rapa*). There is good molecular evidence that introgression can occur in the field under natural⁵⁷ and experimental agricultural conditions^{58–60}. In the United Kingdom, there have been extensive field surveys looking for natural hybridization and introgression between canola and wild *B. rapa*. Data indicate that hybridization is rare (0.4%), which leads to

Box 2 | Transgenes at risk of being introgressed from crops to wild relatives

The influence of transgenesis on wild populations is dependent on the transgene, crop and weed system. Similar to other crop genes, transgenes can be divided into categories on the basis of their detrimental effects: neutral in the natural environment, detrimental, or variable depending on the weediness of the recipient species, the degree of natural biological control and the relative selective advantage the gene provides.

Transgenes that have a neutral effect on fitness might spread in natural populations through genetic drift, but would have no subsequent effect on fitness (for example, marker genes such as *nptII*). Genes with detrimental effects are selected against in the natural environment and will not spread. Many of the traits that are associated with crop domestication fall into this last category, including transgenes for male sterility, altered fibre quality and changes in lignin biosynthesis¹⁰¹. Transgenes that produce herbicide and pest resistance will vary in their fitness potential, depending on the invasiveness of the recipient species and the level of natural control. Genes for viral, fungal and pest resistance fall into a group the incorporation of which into natural populations could increase fitness if the pest controls natural populations (such as *Bt* genes^{78,102}). Herbicide-resistance genes fall into a separate category because they are selectively neutral in the natural environment, although if they were incorporated into already weedy species they could abolish a valuable method of control (for example, glyphosate resistance). Transgenes that change the environmental tolerance of a species or alter its patterns of growth and development could result in notable adaptive shifts and have important effects on fitness; for example, stress tolerance, such as cold or salt tolerance, could extend the habitat range of the recipient wild species.

The following features of a transgene increase the likelihood of its introgression into a wild relative: dominance, absence of association with deleterious crop alleles or traits, location on a shared genome, location on a homologous versus a HOMEOLOGOUS CHROMOSOME, or location on non-rearranged chromosomes compared with the wild recipient.

ECOTYPE

A genetic variety of a single species that is adapted for local ecological conditions.

HOMEOLOGOUS CHROMOSOME

A partially homologous chromosome, which usually indicates some original ancestral homology.

the conclusion that introgression would be a slight risk⁶¹. However, the first glyphosate-tolerant weed × crop hybrids have been discovered⁶⁰ in commercial canola fields in one region of Canada where herbicide-tolerant canola was recently introduced. Conceivably, introgressed herbicide-tolerant *B. rapa* is in the process of evolution and might persist as free-living populations (S. I. Warwick, unpublished observations).

Sunflower (*H. annuus*) has an important wild relative of the same species and is sexually compatible with other wild *Helianthus* species. There is ample molecular evidence for intraspecific introgression between crop and weedy sunflowers^{62,63}. Indeed, crop sunflower genes have introgressed into *H. petiolaris*⁶⁴.

High risk crops. Sorghum (*Sorghum bicolor*) will hybridize with wild populations of the same species, the noxious weed johnsongrass (*Sorghum halepense*), *Sorghum almum* and *Sorghum propinquum*. There is good molecular evidence for introgression in wild sorghum⁶⁵, johnsongrass^{65,66} and *S. almum*⁶⁶. Johnsongrass is considered to be one of the worst weeds worldwide, so it would be unwise to genetically modify sorghum for herbicide tolerance or other fitness-enhancing traits.

Proceed with caution. In summary, the risk of crop-to-wild introgression must be analysed on a case-by-case basis. Assuming co-occurrence, crops in which the recipient wild species shows weedy traits and/or the crop species is weedy are likely to be most problematic. Many crops are 'safe' to engineer for most traits. Others must be assessed on a case-by-case basis. Some should be avoided

or approached with caution (TABLE 1). Because of the desire to avoid new weed problems, which might be exacerbated by the transfer of herbicide tolerance, this trait should be used with care (BOX 2).

Will transgenes introgress into wild relatives? At least two separate issues must be considered when evaluating the effects of transgenes (BOX 2) through introgression. The first is that transgenes might increase weediness and therefore affect our ability to control weeds in agriculture^{67,68}. In particular, the fear is that species that are already extensively weedy will benefit from engineered herbicide tolerance in which there will be uniformly high selective pressures for fixation in crop fields. In sorghum, canola, wheat, sunflower and other crops with weedy wild relatives, if introgression is possible it will occur when herbicide tolerance is introduced into the crop. However, this should only happen in habitats in which the herbicide is applied.

The second issue is that transgenes might increase the fitness and competitiveness of wild relatives of crops in natural ecosystems⁶⁹. Transgenes that have the potential to do this include those that confer insect and disease resistance, drought and salt tolerance, and a suite of other fitness-enhancing traits that could be important in natural habitats. Selective pressures on these genes will vary greatly depending on the invasiveness of the recipient species and specific levels of natural control. Selection should favour these genes less than those for herbicide tolerance in herbicide-prone areas, but perhaps more than naturally occurring genetic variants that influence such traits. What degree of certainty can be assigned to the introgression of these transgenes to other free-living species? The extent of sexual compatibility of the crop and its wild relative, and of selection on the transgene in the wild habitat, are important factors that have been discussed frequently. However, there are further barriers to transgene introgression that also need to be considered.

Transgenic crops and sexually compatible wild plants must grow near one another and have overlapping flowering times for introgression to occur. Also, F₁ hybrids must persist for at least one generation and be sufficiently fertile to produce backcross (BC₁) hybrids (FIG. 1). Perhaps the most important barriers are genetic. The transgene must have a selective advantage for the wild relative that is greater than the sum of any selective disadvantages of loci that are in LD with the crop transgene locus (FIGS 2,3). Finally, backcross generations to the wild relative must progress to the point at which the transgene is incorporated into the genome of the wild relative.

Hybrid-zone theory predicts that advantageous alleles will cross species barriers more readily than neutral markers, such as molecular markers^{3,17,70}. As already discussed, at least some transgenes will be advantageous and therefore should be favoured in wild plants. Increased adaptation might be reflected in increases in fecundity (or other measures of fitness) in the wild relative. However, it is not yet clear if such fitness changes will result in increased invasiveness of

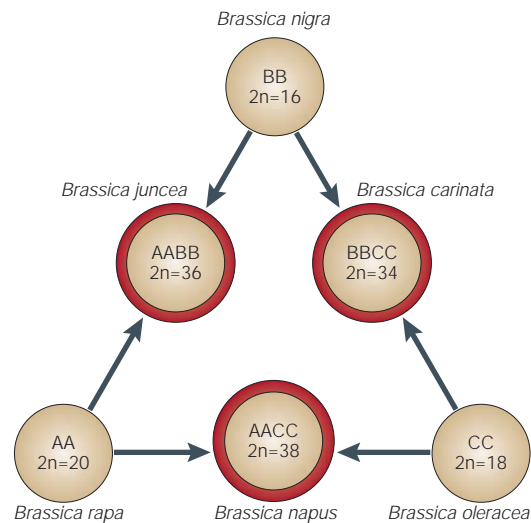


Figure 3 | Genomic relationships among six crop species of Brassica. This figure illustrates the often-complex origins of crop species, many of which are hybrids that contain several genomes. The genomic relationships are represented by a 'triangle of U'¹⁰⁵. Three diploid species are shown (*Brassica rapa*, *Brassica nigra* and *Brassica oleracea*), which represent the AA, BB and CC genomes, respectively. Also shown are three tetraploid species (*Brassica carinata*, *Brassica juncea* and *Brassica napus*), which are hybrid combinations of the basic genomes. Diploid chromosome number (2n) is shown.

the wild relatives of crops or lead to their ecological release. Studies are underway in several systems to address this issue (for example, REF. 71), and early data indicate that serious ecological consequences have not been observed.

As a result of LD, other genes, such as those that control domestication traits, might be co-transferred with transgenes. Although limited information is available on the genetic location of these domestication traits^{72,73}, if transgenes were associated with genomic loci that were historically subject to high levels of LD or located in genomic regions that were rich in domestication genes, this could be a barrier to introgression.

Decreasing or preventing introgression

Although studies of natural introgression and crop breeding are useful in understanding introgression overall, transgene type and transgene genomic placement are likely to be important determinants of introgression rates and the field-level consequences of introgression. Although physical-containment procedures, such as isolation distances⁷⁴ and border rows⁷⁵, have most often been discussed as solutions, biotechnology that focuses on the properties of transgene constructs and their placement in the genome should be able to increase the barriers to introgression.

Transgene placement and the use of LD. Although, at present, the location of transgene insertion in a crop genome is generally random, in future a more targeted approach that takes advantage of LD and/or genomic differences between crops and wild relatives could be used. Transgenes could be inserted into a region of the crop genome that is unlikely to be introgressed. For example, there is great variation in the amount of recombination in different parts of the sunflower¹⁶, *Arabidopsis*⁷⁶, corn²⁷ and cotton⁷⁷ genomes. Presumably, linkage groups that are rarely transferred during recombination would be ideal targets for the location of transgene insertion. Most crop species probably have regions of their genomes that rarely recombine. As more genomic information is gathered, transgenes should be targeted to such regions as a barrier to introgression in a wide range of species. Similarly, when the transgenic crop of interest is POLYPOID (for example, canola, cotton, rice and wheat) (FIG. 3), transgenes could be placed on one of the genomes that is not found in the wild relative of concern. For example, transgene placement on the C genome for canola (AACC) has been proposed as a means to mitigate introgression rates into *B. rapa* (AA)^{78,79}. However, this strategy would only be effective if recombination between the two genomes is infrequent, which is not always the case (FIG. 4).

Using the same principle, another strategy would be to physically link or even fuse transgenes with genes that confer domestication properties that lead to a fitness disadvantage in wild relatives⁸⁰. For example, a fitness-enhancing transgene could be flanked by genes that lead to decreased seed dormancy or seed shattering. In a transgenic construct, the likelihood of crossing over is similar to that of the trait being inactivated by mutation:

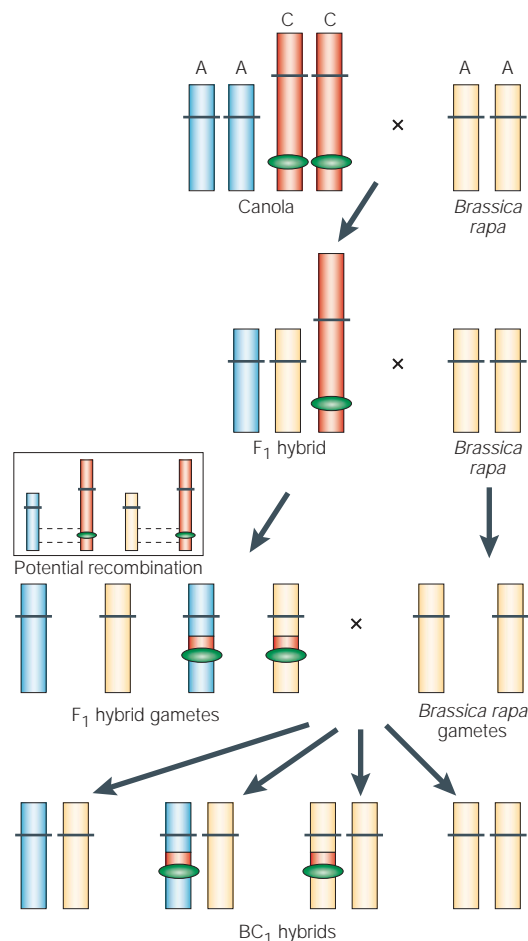


Figure 4 | Specific placement of transgenes in polyploid genomes might not be a barrier to introgression. In the case illustrated, the polyploid canola crop *Brassica napus* (AACC) contains a transgene (green oval) that is located on a genome (C) that is absent in the diploid wild relative *Brassica rapa* (AA). Hybrids that are produced from the initial cross are triploid (AAC). Various gametic types might be produced during meiosis, some of which do not contain the C genome chromosome (with the transgene). Recombination events between the C and A genomes during gametic formation might transfer the transgene to the A genome of the crop, which is shared with the wild relative. This example illustrates that placing a transgene on the genome in a polyploid crop species that is not shared with a specific wild relative does not guarantee that it cannot be transferred. In this case, meiotic recombination between homeologous chromosomes has led to BC₁ hybrids in which the transgene is segregating.

10^{-5} to 10^{-7} (REF. 80). If the transgene were flanked by two domestication genes, the loss of both through mutational inactivation or crossing over would be extremely unlikely ($\sim 10^{-12}$)⁸⁰. Even these rare cases of the transgene escaping from its fitness-reducing 'guards' through recombination could easily be prevented if the fitness-enhancing transgenes and the domestication genes were directly linked through FUNCTIONAL TRANSLATIONAL FUSION. All three methods of using LD to help contain transgenes to the crop species of interest could be combined.

POLYPOID

The genomic state of having three or more sets of homologous chromosomes (for example, tetraploid organisms, which have four sets of chromosomes).

FUNCTIONAL TRANSLATIONAL FUSION

The in-frame chimaera of two or more genes that gives rise to a single chimeric protein.

Non-nuclear genome engineering. Most strategies that introduce exogenous genes to crop species focus on inserting the transgene into the nuclear genome. Only a few species in the Solanaceae and other taxa have proven to be amenable to placement of the transgene in an organellar genome. As organelles are primarily maternally inherited, and so are not present in the highly dispersible pollen grains, chloroplast engineering has been proposed as a mode of transgene containment⁸¹.

However, chloroplast transformation is far from being a universal containment strategy as the biparental inheritance of plastids has been widely documented^{82–84}. This strategy, and others that are based on preventing the occurrence of transgenic pollen (see below), are only effective if the transgenic plant does not serve as the female parent in hybridization and subsequent backcrossing, which is not always the case⁸⁵. Transfer of the transgene from the chloroplast to the nucleus is also a potential problem. A recent study calculated a direct intracellular transfer rate of 1 event per 16,000 pollen grains in tobacco⁸⁶. However, these data indicate that transfer events occur only rarely and, regardless, it is unlikely that the transferred DNA fragment would function in the nucleus because of differences between nuclear and plastid gene regulation and expression. Field-based research is needed to test the efficacy of a chloroplast transformation-based transgene-containment system.

Reduced plant fertility as a limit to gene flow. Several male-sterility systems that target pollen have been developed to limit or control gene flow. In one system, which has been commercialized in canola, the *barnase* gene under the control of a tapetum-specific promoter causes pollen or male sterility. Barnase, which is a cell toxin, kills the cells that would otherwise lead to the production of viable pollen by flowers. Fertility might be restored using the *barstar* gene, which encodes a *barnase*-specific inhibitor⁸⁷. Another system relies on constitutive expression of the *Agrobacterium rolC* gene for sterility. When plants that express this gene are crossed with plants with *rolC* in an antisense orientation, fertility can be restored⁸⁸. An alternative strategy relies on site-specific recombination to remove the transgenes from pollen (REF. 89 and Y. Li, manuscript in preparation).

Controlling the fertility of embryos and seeds, although generally regarded as a better target than pollen fertility, has been a more controversial measure for limiting gene flow. A patent was issued in 1998 that described methodology to chemically control genes that effectively prevent seed germination. The technology, unfortunately dubbed 'Terminator,' was not further developed because of a highly publicized controversy⁹⁰. Strategies that are based on the control of embryo and/or seed fertility have now become known as GENE USE-RESTRICTION TECHNOLOGY (GURT)⁹¹. One example of such an approach is that in which a blocking sequence — a recoverable block function (RBF) — that prevents some essential physiological function in the host plant is inserted, which results in reduced embryo viability⁹¹. As the name

implies, the effects of the block can be removed to recover viability.

The repressible seed-lethal (SL) system is another GURT⁹². In the SL system, the transgene is linked to genes that control seed germination (but do not alter the ability of the crop to set seed) in a repressible construct. When plants that contain this construct are crossed with those that are transgenic for a repressor construct, seed lethality is turned off and seed germination is normal. So, if lethality and repressor constructs are separated during mating, the resultant seeds do not germinate. However, this system is only effective when one of the two genes is present in a haploid gamete. This is not the case for all interspecific hybrids. For example, unreduced gametes are frequently observed in interspecific hybrids between canola and other *Brassica* species⁹⁴.

Future research needs

We still do not have a comprehensive understanding of the risks of transgene introgression. We know that genes can be naturally introgressed between different species, albeit at generally low frequencies and over long periods of time. However, government regulators of transgenic plants are interested in specific transgenes, transgenic events, crops and wild relatives, in time spans of tens of years and beyond. Also, risks must be measured against benefits. To better understand the risks of introgression, and to manage it and its consequences, we propose that the areas discussed below should be the focus of our research efforts.

GURT development. Biotechnological innovations will be important in limiting the degree of transgene flow from GM plants. Public research funds should be assigned to develop effective GURT systems for transgene containment. A combination of pollen- and embryo-specific systems is likely to be most effective in decreasing the risk of transgene escape.

Molecular mapping and surveys. The increasing availability of molecular markers and data on crop plant genomes should allow a more sophisticated understanding of introgression among differentiated plant populations. The absence of molecular evidence of introgression between many crops and their wild relatives could reflect either the lack of interest in publishing negative results or the dearth of appropriate research. Regardless, carrying out such research, especially in the developing world and centres of crop origin, is crucial, as is its publication. Crop-specific markers should be intensively surveyed in populations of the wild relatives of crop plants in the areas of greatest concern. Also, a comparative-mapping approach¹³ could highlight regions of the genome that are less prone to introgression and are therefore candidates for transgene insertion. Similarly, a better understanding of the genomic distribution and function of genes that are related to domestication in crops would allow us to better assess their potential to be used in transgene containment through, for example, forced introgression experiments.

GENE USE-RESTRICTION TECHNOLOGY (GURT). A biotechnological tool that controls embryo viability whereby the addition of a recoverable blocking sequence prevents some essential physiological function in a host plant and can be inducibly removed to recover viability.

Box 3 | 'Pharm farm' crops

The economic benefits of the production of pharmaceuticals in transgenic plants are great, but the containment of genes that express pharmaceutical products poses important challenges. Gene flow and volunteerism that involve pharmaceutical crops are issues that could have direct consequences for human health, and recent actions by regulatory agencies and biotechnology firms reflect the importance and emphasis on containment. In 2002, ProdiGene, Inc. (College Station, Texas, United States) was at the centre of a widely publicized debate that involved the safe containment of pharmaceutical producing crops¹⁰³. The case concerned volunteer transgenic corn plants that were grown the following year in a field planted with soybean. Possible contact between the volunteer corn and soybean plants caused the United States Department of Agriculture (USDA) to impound all soybeans that could have been affected. In December of that year, the USDA reached an agreement with ProdiGene regarding violations of the Plant Protection Act (PPA) arising from Nebraska and Iowa field tests¹⁰⁴. In the settlement, ProdiGene neither admitted nor denied violations, but accepted a \$250,000 civil penalty and agreed to pay the costs of 500,000 bushels of soybeans and of cleaning all facilities and equipment. On one hand, this example shows that biotechnology safeguards are in place, as none of the soybeans in question reached the human food supply. On the other hand, this case illustrates that gene flow and the biology of 'pharm farm' plants is of central importance in regulation and containment. Indeed, it is foreseeable that pharmaceuticals might better be produced in non-commodity plants that are grown in isolation or under glass.

Transgene/domestication effects. To help understand the transfer of adaptations between differentiated plant populations and the subsequent consequences on fitness and competition, we suggest that fitness-enhancing genes, such as those that confer insect resistance, could be combined with domestication genes and used in appropriate experimental crop/wild-relative systems. These experiments could be done in at least two different ways: many single-copy transgenes inserted in different regions of the genome(s) could be compared, or transgene fusions or linkages with domestication genes might be used directly.

We favour an approach in which transgenic and non-transgenic weeds compete against crops⁹⁴. A decreased yield from a crop that is competing with a transgenic weed indicates that introgression of the transgene from crop to weed would result in a more problematic weed. However, if hybrids and backcrosses that contain a transgene cannot effectively compete with wild-type weedy plants of the same taxon then it is likely that introgression will not occur.

Crop-breeding technologies using markers, marker-assisted selection and field competition should also explain how transgenes might be introgressed into wild relatives. It is important to use commercially relevant transgenes and crop/wild-relative models to provide useful and predictive results. Growing and testing transgenic plants in the field is also crucial for predicting the consequences of releasing transgenes into the environment.

Perspectives

The idea that gene flow between transgenic crops and their wild relatives automatically leads to the creation of superweeds has become widespread, despite the lack of scientific studies to support it. By contrast, introgression and the consequences of introgression have been extensively studied in natural and agricultural systems. So, although this review has focused on the key issue of introgression between crops and their wild relatives, gene flow from genetically modified crops

will continue to receive much attention, even if it is only to other crops. This point is highlighted by recent concerns about the gene flow of pharmaceutical-associated transgenes from transgenic to non-transgenic crop plants (BOX 3).

With respect to the important issue of introgression from GM crops, we recommend that large-scale genetic modification should be avoided for high-risk crops in which introgression is well documented. However, our assessment is that, in most cases, the risks and benefits of transgenes should be considered on a case-by-case basis. For example, it is hard to imagine strategies for transgene containment that are based on co-location with domestication genes or GURT that would be effective in preventing the introgression of transgenes that confer herbicide tolerance. The strong selective advantage such genes provide would only need a single instance of containment failure and transgene escape for the gene to spread and become fixed in a sexually compatible wild relative. All other traits should be considered potentially safe to engineer into most crops.

Although schemes that classify crops into different risk categories for transgene introgression (such as those suggested here) are useful, they cannot be anything other than rough guides that are subject to reassessment for specific crop/transgene combinations when new evidence is found. For example, recently it was shown that transgenic sunflowers (a moderate risk crop in our scheme) were unlikely to transfer their disease-tolerance transgene to wild sunflowers⁹⁵. The oxalate oxidase (*OxOx*) transgene did not increase fitness in *BC₃* sunflowers and so would not be selected for in free-living environments. There is still much research to be done before we fully understand the risks that are associated with introgression from transgenic crops. Nevertheless, a suite of increasingly innovative transgene-containment strategies, coupled with a growing knowledge of the genetics and ecology of introgression among plants, indicate that the environmental risks can be minimized. This is encouraging and, indeed, vital because GM crops are here to stay.

1. Quist, D. & Chapela, I. H. Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico. *Nature* **414**, 541–543 (2001).
2. Editorial note. *Nature* **416**, 600 (2002).
3. Rieseberg, L. H. & Wendel, J. F. In *Hybrid Zones and the Evolutionary Process* (ed. Harrison, R. G.) 70–109 (Oxford Univ. Press, New York, 1993).
- This extensive review showed how the use of molecular markers has transformed our understanding of introgression.**
4. Metz, M. & Fütterer, J. Suspect evidence of transgenic contamination. *Nature* **416**, 600–601 (2002).
5. Stebbins, G. L. The inviability, weakness, and sterility of interspecific hybrids. *Adv. Genet.* **9**, 147–215 (1958).
6. Anderson, E. *Introgressive Hybridization* (Wiley, New York, 1949).
- This book was the first to recognize the evolutionary implications of introgressive hybridization, develop methods for its analysis and provide experimental studies of introgression.**
7. Heiser, C. B. Introgression re-examined. *Bot. Rev.* **39**, 347–366 (1973).
8. Grant, V. *Plant Speciation* 2nd edn (Columbia Univ. Press, New York, 1981).
9. Abbott, R. J. Plant invasions, interspecific hybridization and the evolution of plant taxa. *Trends Ecol. Evol.* **7**, 401–405 (1992).
10. Ellstrand, N. C. & Schierenbeck, K. Hybridization as a stimulus for the evolution of invasiveness in plants? *Proc. Natl Acad. Sci. USA* **97**, 7043–7050 (2000).
11. Abbott, R. J., James, J. K., Milne, R. I. & Gillies, A. C. M. Plant introductions, hybridization and gene flow. *Phil. Trans. R. Soc. Lond. B* **358**, 1123–1132 (2003).
12. Rieseberg, L. H. & Brunfeldt, S. J. In *Molecular Systematics of Plants* (eds Soltis, P. S., Soltis, D. E. & Doyle, J. J.) 151–176 (Chapman and Hall, New York, 1992).
13. Rieseberg, L. H., Baird, S. J. E. & Gardner, K. A. Hybridization, introgression, and linkage evolution. *Plant Mol. Biol.* **42**, 205–224 (2000).
- This excellent review makes a convincing case for using molecular map-based approaches for the study of hybridization and introgression.**
14. Harrison, R. G. Hybrid zones: windows on evolutionary process. *Oxford Surv. Evol. Biol.* **7**, 69–128 (1990).
15. Burke, J. M., Voss, T. J. & Arnold, M. L. Genetic interactions and natural selection in Louisiana iris hybrids. *Evolution* **52**, 1304–1310 (1998).
16. Rieseberg, L. H., Whitton, J. & Gardner, K. Hybrid zones and the genetic architecture of a barrier to gene flow between two wild sunflower species. *Genetics* **152**, 713–727 (1999).
17. Barton, N. H. & Hewitt, G. M. Analysis of hybrid zones. *Ann. Rev. Ecol. Syst.* **16**, 113–148 (1985).
18. Kallou, G. & Chowdhury, J. B. *Distant Hybridization of Crop Plants* (Springer, Heidelberg, 1992).
19. Zamir, D. Improving plant breeding with exotic genetic libraries. *Nature Rev. Genet.* **2**, 983–989 (2001).
20. Tanksley, S. D. & McCouch, S. R. Seed banks and molecular maps: unlocking genetic potential from the wild. *Science* **227**, 1063–1066 (1997).
21. Harlan, J. R. *Crops and Man* (Crop Science Soc. of America, Madison, Wisconsin, 1975).
22. Simmonds, N. W. *Principles of Crop Improvement* (Longman, New York, 1979).
23. Baker, H. G. in *The Genetics of Colonizing Species* (eds Baker, H. G. & Stebbins, G. L.) 147–168 (Academic, New York, 1965).
- In this paper, weediness traits are described and compared with domestication traits.**
24. Baker, H. G. The evolution of weeds. *Ann. Rev. Ecol. Syst.* **5**, 1–24 (1974).
25. Charlesworth, D., Charlesworth, B. & Morgan, M. T. The pattern of neutral molecular variation under the background selection model. *Genetics* **141**, 1619–1632 (1995).
26. Cummings, M. P. & Clegg, M. T. Nucleotide sequence diversity at the alcohol dehydrogenase 1 locus in wild barley (*Hordeum vulgare* ssp. *spontaneum*): an evaluation of the background hypothesis. *Proc. Natl Acad. Sci. USA* **95**, 5637–5642 (1998).
27. Remington, D. L. *et al.* Structure of linkage disequilibrium and phenotypic associations in the maize genome. *Proc. Natl Acad. Sci. USA* **98**, 11479–11484 (2001).
28. Haygood, R., Ives, A. R. & Andow, D. A. Consequences of recurrent gene flow from crops to wild relatives. *Proc. R. Soc. Lond. B* **25** July 2003 (doi: 10.1098/rspb.2003).
29. Ellstrand, N. C., Prentice, H. C. & Hancock, J. F. Gene flow and introgression from domesticated plants into their wild relatives. *Ann. Rev. Ecol. Syst.* **30**, 539–563 (1999).
30. Raybould, A. & Gray, A. J. Genetically modified crops and hybridization with wild relatives: a UK perspective. *J. Appl. Ecol.* **30**, 199–219 (1993).
- This much cited review was one of the first to ascribe gene-flow risks for transgenic crops.**
31. Hancock, J. F. A framework for assessing the risk of transgenic crops. *Bioscience* **53**, 512–519 (2003).
32. Gressel, J. & Rotteveel, A. W. Genetic and ecological risks from biotechnologically-derived herbicide-resistant crops: decision trees for risk assessment. *Plant Breed. Rev.* **18**, 251–303 (2000).
33. Shoemaker, R. C. *et al.* Genome duplication in soybean (*Glycine* subgenus *soja*). *Genetics* **144**, 329–338 (1996).
34. Concibido, V. C. *et al.* Introgression of a quantitative trait locus for yield from *Glycine soja* into commercial soybean cultivars. *Theor. Appl. Genet.* **106**, 575–582 (2003).
35. Leroy, A. R., Fehr, W. R. & Cianzio, S. R. Introgression of genes for small seed size from *Glycine soja* into *G. max*. *Crop Sci.* **31**, 693–697 (1991).
36. Simpson, C. E. Use of wild *Arachis* species/introgression of genes into *A. hypogaea* L. *Peanut Sci.* **28**, 114–116 (2001).
37. Garcia, G. M., Stalker, H. T. & Kochert, G. Introgression analysis of an interspecific hybrid population in peanuts (*Arachis hypogaea* L.) using RFLP and RAPD markers. *Genome* **38**, 166–176 (1995).
38. Beebe, S., Toro, C. O., González, A. V., Chacón, M. I. & Debouck, D. G. Wild-weed-crop complexes of common bean (*Phaseolus vulgaris* L., Fabaceae) in the Andes of Peru and Colombia, and their implications for conservation and breeding. *Genet. Res. Crop Evol.* **44**, 73–91 (1997).
39. Johns, M. A. *et al.* Gene pool classification of common bean landraces from Chile based on RAPD and morphological data. *Crop Sci.* **37**, 605–613 (1997).
40. Doebley, J. Molecular evidence for gene flow among *Zea* species. *Bioscience* **40**, 443–448 (1990).
41. Doebley, J. F. Molecular evidence and the evolution of maize. *Econ. Bot.* **44** (Suppl.), 6–27 (1990).
42. Matsuoka, Y. *et al.* A single domestication for maize shown by multilocus microsatellite genotyping. *Proc. Natl Acad. Sci. USA* **99**, 6080–6084 (2002).
43. Doebley, J., Goodman, M. M. & Stuber, C. W. Patterns of isozyme variation between maize and Mexican annual teosinte. *Econ. Bot.* **41**, 234–246 (1987).
44. Majumder, N. D., Ram, T. & Sharma, A. C. Cytological and morphological variation in hybrid swarms and introgressed population of interspecific hybrids (*Oryza rufipogon* Griff. x *Oryza sativa* L.) and its impact on evolution of intermediate types. *Euphytica* **94**, 295–302 (1997).
45. Suh, H. S., Sato, Y. I. & Morishima, H. Genetic characterization of weedy rice (*Oryza sativa* L.) based on morpho-physiology, isozymes and RAPD markers. *Theor. Appl. Genet.* **94**, 316–321 (1997).
46. Akimoto, M., Shimamoto, Y. & Morishima, H. The extinction of genetic resources of Asian wild rice, *Oryza rufipogon* Griff.: a case study in Thailand. *Gen. Res. Crop Evol.* **46**, 419–425 (1999).
47. Song, Z., Baorong, L., Zhu, Y. & Chen, J. Pollen competition between cultivated and wild rice species *Oryza sativa* and *O. rufipogon*. *New Phytol.* **153**, 289–296 (2002).
48. Song, Z. P., Lu, B., Zhu, Y. G. & Chen, J. K. Gene flow from cultivated rice to the wild species *Oryza rufipogon* under experimental field conditions. *New Phytol.* **157**, 657–665 (2003).
49. Kanazawa, A., Akimoto, M., Morishima, H. & Shimamoto, Y. Inter- and intra-specific distribution of *Stowaway* transposable elements in AA-genome species of wild rice. *Theor. Appl. Genet.* **101**, 327–335 (2000).
50. Jenczewski, E., Prosperi, J. M. & Ronfort, J. Differentiation between natural and cultivated populations of *Medicago sativa* (Leguminosae) from Spain: analysis with random amplified polymorphic DNA (RAPD) markers and comparison to allozymes. *Mol. Ecol.* **8**, 1317–1330 (1999).
51. Jenczewski, E., Prosperi, J. M. & Ronfort, J. Evidence for gene flow between wild and cultivated *Medicago sativa* (Leguminosae) based on allozyme markers and quantitative traits. *Amer. J. Bot.* **86**, 677–687 (1999).
52. Bartsch, D. *et al.* Impact of gene flow from cultivated beet on genetic diversity of wild sea beet populations. *Mol. Ecol.* **8**, 1733–1741 (1999).
53. Arnaud, J.-F., Viard, F., Delescluse, M. & Cuguen, J. Evidence for gene flow via seed dispersal from crop to wild relatives in *Beta vulgaris* (Chenopodiaceae): consequences for the release of genetically modified crop species with weedy lineages. *Proc. R. Soc. Lond. B* **270**, 1565–1571 (2003).
54. Seefeldt, S. S., Zemetra, R., Young, F. L. & Jones, S. S. Production of herbicide-resistant jointed goatgrass (*Aegilops cylindrica* x wheat (*Triticum aestivum*) hybrids in the field by natural hybridization. *Weed Sci.* **46**, 632–634 (1998).
55. Wang, Z., Zemetra, R. S., Hansen, J. & Mallory-Smith, C. A. The fertility of wheat x jointed goatgrass hybrid and its backcross progenies. *Weed Sci.* **49**, 340–345 (2001).
56. Morrison, L. A., Crémière, L. C. & Mallory-Smith, C. A. Infestations of jointed goatgrass (*Aegilops cylindrica*) and its hybrids with wheat in Oregon wheat fields. *Weed Sci.* **50**, 737–747 (2002).
57. Hansen, L. B., Siegmund, H. R. & Jørgensen, R. B. Introgression between oilseed rape (*Brassica napus* L.) and its weedy relative *B. rapa* L. in a natural population. *Gen. Res. Crop Evol.* **48**, 621–627 (2001).
58. Halfhill, M. D., Richards, H. A., Mabon, S. A. & Stewart, C. N. Expression of GFP and Bt transgenes in *Brassica napus* and hybridization with *Brassica rapa*. *Theor. Appl. Genet.* **103**, 659–667 (2001).
- In this study, an in vivo monitoring system was used to observe introgression and gene expression through the visual marker green fluorescent protein (GFP)**
59. Halfhill, M. D., Millwood, R. J., Weissinger, A. K., Warwick, S. I. & Stewart, C. N. Additive transgene expression and genetic introgression in multiple GFP transgenic crop x weed hybrid generations. *Theor. Appl. Genet.* (DOI 10.1007/s00122-003-1397-7).
60. Warwick, S. I. *et al.* Hybridization between *Brassica napus* L. and its wild relatives: *B. rapa* L., *Raphanus raphanistrum* L., *Sinapis arvensis* L., and *Erucastrum gallicum* (Willd.) O. E. Schulz. *Theor. Appl. Genet.* **107**, 528–539 (2003).
- The first evidence of transgene escape to a wild relative from a commercially released GM crop.**
61. Scott, S. E. & Wilkinson, M. J. Transgene risk is low. *Nature* **393**, 320 (1998).
62. Linder, C., Taha, I., Seiler, G., Snow, A. & Rieseberg, L. Long-term introgression of crop genes into wild sunflower populations. *Theor. Appl. Genet.* **96**, 339–347 (1998).
63. Whitton, J., Wolf, D. E., Arias, D. M., Snow, A. A. & Rieseberg, L. H. The persistence of cultivar alleles in wild populations of sunflowers five generations after hybridization. *Theor. Appl. Genet.* **95**, 33–40 (1997).
64. Rieseberg, L. H., Kim, M. J. & Seiler, G. J. Introgression between the cultivated sunflower and a sympatric wild relative, *Helianthus petiolaris* (Asteraceae). *Int. J. Plant Sci.* **160**, 102–108 (1999).
65. Renganayaki, K., Amirthadevaranthinam, A. & Sadasivam, S. Species relationship and hybrid identification in sorghum using RAPD, protein and isozyme techniques. *J. Genet. Breed.* **54**, 117–124 (2000).
66. Paterson, A. H., Schertz, K. F., Lin, Y. R., Liu, S.-C. & Chang, Y.-L. The weediness of wild plants: molecular analysis of genes influencing dispersal and persistence of Johnsongrass, *Sorghum halepense* (L.) Pers. *Proc. Natl Acad. Sci. USA* **92**, 6127–6131 (1995).
- This paper describes an early approach to the analysis of the genomic basis of weediness.**
67. Darmency, H. The impact of hybrids between genetically modified crop plants and their related species: introgression and weediness. *Mol. Ecol.* **3**, 37–40 (1994).
68. Warwick, S. I., Beckie, H. J. & Small, E. Transgenic crops: new weed problems for Canada? *Phytoprotection* **80**, 71–84 (1999).
69. Snow, A. A. & Palma, P. M. Commercialization of transgenic plants: potential ecological risks. *Bioscience* **47**, 86–96 (1997).
70. Muir, W. M. & Howard, R. D. Fitness components and ecological risk of transgenic release: a model using Japanese medaka (*Oryzias latipes*) *Am. Nat.* **158**, 1–16 (1999).
71. Cummings, C. L. *et al.* Recurrent selection in an experimental sunflower crop–wild system: how well do ecological data predict crop allele persistence. *Ecol. Appl.* **12**, 1661–1671 (2002).
72. Doebley, J. in *Molecular Systematics of Plants* (eds Soltis, P. S., Soltis, D. E. & Doyle, J. J.) 202–222 (Chapman and Hall, New York, 1992).
73. Burke, J. M., Tang, S., Knapp, S. J. & Rieseberg, L. H. Genetic analysis of sunflower domestication. *Genetics* **161**, 1257–1267 (2002).
74. Ingram, J. The separation distance required to ensure cross-pollination is below specified limits in non-seed crops of sugar beet, maize and oilseed rape. *Plant Var. and Seeds* **13**, 181–199 (2000).
75. Morris, W. F., Kareiva, P. M. & Raymer, P. L. Do barren zones and pollen traps reduce gene escape from transgenic crops? *Ecol. Appl.* **4**, 157–165 (1994).
76. Nordborg, M. *et al.* The extent of linkage disequilibrium in *Arabidopsis thaliana*. *Nature Genet.* **30**, 190–193 (2002).
77. Jiang, C. X. *et al.* Multilocus interactions restrict gene introgression in interspecific populations of polyploid *Gossypium* (cotton). *Evolution* **54**, 798–814 (2000).
78. Halfhill, M. D., Millwood, R. J., Raymer, P. L. & Stewart, C. N. Bt-transgenic oilseed rape hybridization with its weedy relative, *Brassica rapa*. *Environmental Biosafety Res.* **1**, 19–28 (2002).
79. Metz, P. L. J., Jacobsen, E., Nap, J. P., Pereira, A. & Stiekema, W. J. The impact on biosafety of the phosphinothricin-tolerance transgene in inter-specific *B. rapa* x *B. napus* hybrids and their successive backcrosses. *Theor. Appl. Genet.* **95**, 442–450 (1997).
80. Gressel, J. Tandem constructs: preventing the rise of superweeds. *Trends Biotech.* **17**, 361–366 (1999).
- This was the first account to recognize the power of using transgene linkage as a means to prevent or decrease introgression.**
81. Daniell, H., Datta, R., Varma, S., Gray, S. & Lee, S. B. Containment of herbicide resistance through genetic engineering of the chloroplast genome. *Nature Biotechnol.* **16**, 345–348 (1998).

82. Corriveau, J. P. & Coleman, A. W. Rapid screening method to detect potential biparental inheritance of plastid DNA and results for over 200 angiosperm species. *Am. J. Bot.* **75**, 1443–1458 (1988).
83. Hagemann, R. In *Cell Organelles* (ed. Hermann, R. G.) 65–96 (Springer, Berlin, 1992).
84. Reboud, X. & Zeyl, C. Organelle inheritance in plants. *Heredity* **72**, 132–140 (1994).
85. Chamberlain, D. & Stewart, C. N. Transplastomics and transgene escape. *Nature Biotechnol.* **17**, 330–331 (1999).
86. Huang, C. Y., Ayliffe, M. A. & Timmis, J. N. Direct measurement of the transfer rate of chloroplast DNA into the nucleus. *Nature* **422**, 72–76 (2003).
87. DeBlock, M. & Debrouwer, D. Engineered fertility control in transgenic *Brassica napus* L.: histochemical analysis of anther development. *Planta* **189**, 218–225 (1993).
88. Schmuelling, T., Roehrig, H., Pliz, S., Walden, R. & Schell, J. Restoration of fertility by antisense RNA in genetically engineered male sterile tobacco plants. *Mol. Gen. Genet.* **237**, 385–394 (1993).
89. Keenan, R. J. & Stemmer, W. P. C. Nontransgenic crops from transgenic plants. *Nature Biotechnol.* **20**, 215–216 (2002).
90. Masood, E. Monsanto set to back down over “terminator” gene? *Nature* **396**, 503 (1998).
91. Kuvshinov, V., Koivu, K., Kanerva, A. & Pehu, E. Molecular control of transgene escape from genetically modified plants. *Plant Sci.* **160**, 517–522 (2001).
92. Scherthamer, J. P., Fabijanski, S. F., Arnison, P. G., Racicot, M. & Robert, L. S. Control of seed germination in transgenic plants based on the segregation of a two-component genetic system. *Proc. Natl Acad. Sci. USA* **100**, 6855–6859 (2003).
93. Heyn, F. W. Analysis of unreduced gametes in the Brassicaceae by crosses between species and ploidy levels. *Z. Pflanzenzüchtg.* **78**, 13–30 (1977).
94. Ramachandran, S., Buntin, G. D., All, J. N., Raymer, P. L. & Stewart, C. N. Intraspecific competition of an insect-resistant transgenic canola in seed mixtures. *Agron. J.* **92**, 368–374 (2000).
95. Burke, J. M. & Rieseberg, L. H. Fitness effects of transgenic disease resistance in sunflowers. *Science* **300**, 1250 (2003). **This is the most comprehensive study, so far, of the consequences of the introgression of a fitness-associated transgene into a wild relative of a crop. This showed that a disease-resistance transgene would not increase the fitness of a wild plant.**
96. Arnold, M. L. Natural hybridization as an evolutionary process. *Ann. Rev. Ecol. Syst.* **23**, 237–261 (1992).
97. Arnold, M. L. & Bennett, B. D. in *Hybrid Zones and the Evolutionary Process* (ed. Harrison, R. G.) 115–139 (Oxford Univ. Press, New York, 1993).
98. Arnold, M. L. *Iris nelsonii* (Iridaceae): origin and genetic composition of a homoploid hybrid species. *Amer. J. Bot.* **80**, 577–583 (1993).
99. Bennett, B. D. & Grace, J. B. Shade tolerance and its effect on the segregation of two species of Louisiana *Iris* and their hybrids. *Amer. J. Bot.* **77**, 100–107 (1990).
100. Cruzan, M. B. & Arnold, M. L. Assortative mating and natural selection in an *Iris* hybrid zone. *Evolution* **48**, 1946–1958 (1994).
101. Saxena, D. & Stotzky, G. Bt corn has a higher lignin content than non-Bt corn. *Am. J. Bot.* **88**, 1704–1706 (2001).
102. Snow, A. A. *et al.* A Bt transgene reduces herbivory and enhances fecundity in wild sunflowers. *Ecol. Appl.* **13**, 279–286 (2003).
103. Hoag, H. Tougher rules aim to prevent gene flow into crops. *Nature* **422**, 103 (2003).
104. United States Department of Agriculture. USDA announces actions regarding Plant Protection Act violations involving Prodigene Inc. News Release No. 4098.02 [online], (cited 05 Sep. 2003). <<http://www.usde.gov/news/releases/2002/12/0498.htm>> (2002).
105. N. U. Genomic-analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. *Jpn. J. Bot.* **7**, 389–452 (1935). **This impressive deductive investigation of the polyploid genomic relationships among Brassicas was made long before the development of molecular tools. The author used the word ‘genomic’ 60 years before the modern genomics revolution and his conclusions have stood the test of time.**
106. Aldrich, P. R. & Doebley, J. Restriction fragment length variation in the nuclear and chloroplast genomes of cultivated and wild Sorghum bicolor. *Theor. Appl. Genet.* **85**, 293–302 (1992).
107. Gómez, M. I. *et al.* Tetraploid nature of *Sorghum bicolor* (L.) Moench. *J. Heredity* **89**, 188–190 (1998).
108. Santoni, S. & Berville, A. Evidence for gene exchanges between sugar beet (*Beta vulgaris* L.) and wild beets: consequences for transgenic sugar beets. *Plant Mol. Biol.* **20**, 578–580 (1992).
109. Desplanque, B. *et al.* Genetic diversity and gene flow between wild, cultivated and weedy forms of *Beta vulgaris* L. (Chenopodiaceae) assessed by RFLP and microsatellite markers. *Theor. Appl. Genet.* **98**, 1194–1201 (1999).

Acknowledgements

We are grateful for support from the United States Department of Agriculture Biotechnology Risk Assessment Grants Program and the University of Tennessee Institute of Agriculture.

Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.