

Ecological and Biological Aspects of the Impacts of Transgenic Maize, Including Agro-Biodiversity

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EXECUTIVE SUMMARY

State of Knowledge: Areas of Consensus

Mexico as the center of origin of maize diversity: Present state of the biodiversity of local races and wild teosintes

The maize plant is an annual Graminae, *Zea mays* ssp. *mays* L., and is the grain with the highest production levels in the world. In Mexico, it is the most important cultivar in terms of production area devoted to it (more than 7 million hectares in 2001, including one million hectares of hybrid corn) and second in terms of gross volume of production (18.6 million tons in 2001). Mexico is the center of origin and diversity of the races of maize, with more than 60 recognized up to now and with many more subraces and local varieties. Mexico is also the home of various wild relatives of maize, the teosintes, among which we find the putative ancestor of maize and the perennial teosinte. Various of these teosintes and races of maize are endangered because of recent changes in the use of land and overall environmental degradation. It is of outmost importance to conserve these species, independently of the possible impact of transgenic varieties.

Environmental effects due to the release of transgenic maize varieties

In ecological terms, the most widely discussed risks of the release of transgenic varieties into the environment are those derived from the unintentional movement of transgenes into wild and cultivated populations. We will summarize here the data available on two main aspects of this issue for the case of maize in Mexico: (1) The possibility of unintentional introgression (entrance and persistence of a transgene) from a transgenic to a wild or cultivated non-transgenic population in Mexico. (2) The biological consequences of such introgression.

Gene flow

Gene flow from maize to teosinte (*Z. m.* ssp. *mexicana*) in Mexico occurs at low rates per generation when plants grow in proximity, but alleles from cultivated varieties have introgressed into teosintes over generations. Traits from cultivated improved commercial varieties of maize have also introgressed into local varieties when both are grown several hundreds of kilometers apart. Therefore, once transgenic varieties grow in proximity to local wild and cultivated varieties, gene flow and introgression of transgenes into these local varieties is expected. Furthermore, the individual teosinte and maize plants that carry newly introgressed transgenes can function as natural bridges for introgression into other varieties. Also, the exchange of seeds among peasants in Mexico can cause the areas in which gene flow and introgression of transgenes occur to be larger than would be expected if gene flow via pollen were the source of transgenes coming into native populations.

Consequences of gene flow

Persistence of transgenes after gene flow

Once gene flow has occurred, the permanence of a transgene will depend on the fitness effects of its expression. If these effects are neutral, the transgene will remain in the population at a frequency that will depend on the gene flow frequency. If the transgene

increases fitness, then it will increase until fixation, but if it decreases fitness, then it will decrease until it disappears from the population. We summarize information on the expected effects of introgression in wild and/or native varieties of maize in Mexico of the transgenes that are the object of deregulated biotechnological developments in the United States for this crop.

Weed evolution: resistance to herbicides and pests

In the case of maize, the introduction of herbicide or pest resistance can be problematic if modified plants are introduced into places where teosinte is a weed and it is controlled with the herbicide to which the transgene is resistant, or if it was naturally controlled by the pests that are affected by the expression of the introduced transgene. Both cases would render it hard to control weed teosintes. In neither case would one expect weeds to evolve from introgression into cultivated maize varieties.

Male sterility

Genes that cause male sterility in maize are not expected to remain in the populations or to cause any detrimental effect in local wild or cultivated maize populations in Mexico.

Gene erosion

In open-pollinated species like maize, recombination would guarantee that the detrimental effects on genetic variation, due to transgene introgression, would be restricted to very small portions of the genome.

Evolution of resistant insects and new pests

It is clear that new, hard-to-control insect pests can arise if flow of *Bt* genes that confer resistance to lepidopteron pests, occurs in populations that are not subject to regulation.

Unexpected effects

Unexpected effects can occur, both due to impacts on species that are not the target of biotechnological developments and due to pleiotropic effects of transgenes in the transformed plant. The first type of unexpected effects can be exemplified by the possible impact of Bt protein on the larvae of the monarch butterfly in Mexico. This has been the most widely studied case. The most recent data suggest that the effects expected in the field are very mild, due to the low levels of Bt expression in the pollen on which the monarch larvae feed. In the second case, pleiotropic effects of Bt expression have been documented in experimental conditions. In any case, it is important to undertake long-term environmental monitoring to document novel unexpected effects of any deregulated transgene.

State of Knowledge: Areas of Debate

Mexico as the center of origin of maize diversity: Present state of the existent biodiversity of local races and wild teosintes

There is debate on the number and genealogical relationships among local races of maize in Mexico. It is important to solve this issue for any analysis on the possible impact of transgenic varieties.

Environmental effects due to the release of transgenic maize varieties

Gene flow

Gene flow from transgenic varieties into wild relatives has been emphasized. But gene flow from a transgenic cultivar into a nontransgenic one is equally important. Since the publication of the paper by Quist and Chapela (2001) in *Nature*, there has been a heated debate around the possibility that transgenes have already introgressed into local maize varieties of Oaxaca. Recent data from two Mexican laboratories hired by Conabio and INE in Mexico suggest that transgenes are present in the genomes of local races of maize collected in Oaxaca and Puebla. New experiments are being done to confirm these preliminary results.

Consequences of gene flow

These are debated and more data and new models are needed to analyze the possible long-term unexpected effects of transgenes once these introgress into wild or cultivated populations for which transgenes were not intended.

Areas in which Unresolved and Controversial Issues Exist: Priority Themes on which More Information and Research Are Needed to Improve Understanding of Risks to Biodiversity

It is of the highest priority to resolve definitely the question of whether transgene flow and introgression have occurred in local varieties of Mexico. Given that gene flow and introgression of transgenes into local maize varieties that are intended for animal or human food are clearly possible, if not a fact, it is particularly worrying that such introgression comes from transgenic lines that express industrial or pharmaceutical biochemicals. These are the object of new developments in maize that are now under field trials in the United States. Gene flow from these transgenic varieties would certainly affect food security and it is of the highest priority to evaluate this possibility and, if necessary, to begin means of contention or to stop this type of developments. Other areas in which it is important to stimulate more research, because of their possible impacts, are: lateral gene transfer and its impact on antibiotic resistance and the evolution of new pathogens; the accumulation of independent DNA in the environment as a possible biotoxic agent; and the possible genomic instability of transgenes.

1 State of Knowledge: Areas of Consensus

The maize plant is an annual Gramineæ, *Zea mays* ssp. *mays* L., that produces what is commonly known as kernels of corn (technically the fruit) on the female infructescence, which is, in turn, known as the maize corn cob. Maize is a versatile cultivated plant that is used for human and animal consumption, in both processed and unprocessed forms. It is the most widely produced grain in the world: the United States is the largest producer, accounting for approximately 40 percent of the grain produced in the world, followed by China, Brazil and Mexico. In Mexico, maize is the most important cultivated plant in terms of area of production (more than seven million hectares in 2001) and the second in terms of gross volume of production (18.6 million tons in 2001).

1.1 Mexico as center of origin of maize diversity: Present state of the existent biodiversity of local races and wild teosintes

All archeological and biological evidence indicates that maize originated in Mexico, probably in western Mexico (Benz 1986) or in the Balsas (Iltis 1987, Doebley 1990) 7,500 to 10,000 years ago (Wang *et al.* 1999, Tenaillon *et al.* 2001). Mexico has contributed at least 50 races of maize, adapted to different climatic and altitudinal conditions that go from 0 up to 2700 meters above sea level (Hernández X. 1985, Sánchez 1993). However, it is likely that there are 60 or more native varieties in Mexico (Dr. B. Benz, personal communication), and a considerable number of subraces and local varieties that have not been characterized yet.

Teosintes or wild maize relatives have played and continue to play an important role in the origin of the cultivated races in Mexico (Welhausen *et al.* 1952, Wilkes 1972, 1977 and 1996, Doebley *et al.* 1984, Smith *et al.* 1985; Hancock 1992) and most of the teosintes are associated with traditional cultivation practices in corn fields (Sánchez and Ordaz 1987, Serratos-Hernández, Islas-Gutierrez and Berthaud, 2001). They are also recognized as closest wild relatives of maize (Doebley and Iltis, 1980; Doebley 1990, Iltis and Doebley 1980). In fact, one of the teosintes is considered the ancestral species to maize: *Zea mays* sub sp. *parviglumis* (Doebley 1990, Iltis 2000). Another is particularly important because it is perennial and could be the source of new genes for introgression into maize to generate perennial cultivated varieties. Besides its importance as the ancestor of maize or as a source of genes for maize breeding programs, teosinte (*Zea mays* L. subsp. *Parviglumis*, Iltis and Doebley) has also the potential to be a forage plant for cows and horses (Miranda *et al.* 2001), although usually it is considered a weed by local peasants. It is also key for traditional agriculture that generally takes place under suboptimal soil conditions where teosintes can grow and are favored as a source of genes for making maize varieties that are also able to grow under these conditions. Figure 1 shows that the geographical distribution of cultivated maize races and teosintes in Mexico overlap in part of the maize distribution range. The levels of maize production are also shown in this figure.

Many of the maize races and most species of teosinte are endangered (Blancas 2001; Sánchez, pers. com.). Their population sizes and distribution have been affected by general land-use practices, intensive agriculture and urbanization (Wilkes 1996; Sánchez and Ruíz 1996). Therefore, aside from the possible impacts of transgenic maize introduction, it is of

the highest priority to conserve the races of maize and teosinte species in Mexico. Conserving the diversity of maize is a matter of ensuring food security for Mexico and the rest of the world and this will depend on *in situ* as well as *ex situ* programs. Although there are relatively good collections and seed banks containing the Mexican maize races, there are several races that have not been registered. For example, CIMMYT in Mexico has 8264 collections of maize and we know that at least 2000 more are needed in order to have a complete collection of the Mexican races and local varieties in Mexico (Taba, pers. com.). Present teosinte collections are even poorer in scope. For a review on teosintes distribution, conservation in germplasm banks and the regional diversity of these wild maize species in Mexico, see Sánchez *et al.* (1998). Modern improved varieties represent ca. 24% of the genetic diversity of Mexican local maize races (Tenaillon *et al.* 2001; Tenaillon *et al.*, 2002). We know even less about the population level and structure of genetic variation in cultivated and wild maize in Mexico (Blancas 2001).

Biodiversity of cultivated and wild maize is associated with various production systems that are generally found in mosaics of cultivated and natural areas. These range from temperate conifer forests to rainforests with various degrees of conservation. This constitutes the ecological context in which the effects of maize transgene introduction should be evaluated.

1.2 Environmental effects due to the release of transgenic maize varieties

Most discussions on the ecological effects of transgenes have focused on the traits that result from the expression of the genes that are the specific object of biotechnological developments. The main concern has been the possible escape of these transgenes from regulated areas into populations of cultivated and wild populations for which such developments were not intended. Most attention has been devoted to the ecological impacts of gene flow and permanence of transgenes in these populations, although limited data is still available.

In contrast, the ecological risks derived from the process implied in the genetic modification of plants using recombinant DNA techniques have been rarely discussed. Such risks derive from: (a) aspects of the procedures used for genetic transformation of plants and animals that are not well understood, and (b) the hard-to-predict or unexpected effects of all the DNA sequences and plasmids used during genetic transformation, once transgenic organisms are released into the environment. These unexpected and complex effects depend on the spatio-temporal dynamics of the ecological systems. Much more investigation is needed before we can draw conclusions about these types of effects. Therefore, we will discuss these in the last section of this essay. In this section, we will discuss the risks to biodiversity that might arise from the expression of genes in maize that have been the subject of biotechnological developments grown under open field conditions in the United States, in case these should escape into populations for which they were not intended. We will center on the possible consequences of undesired gene flow in maize under Mexican conditions.

1.2.1 Gene flow

Pollen dispersal varies among cultivated or wild species. But within the same species, pollen dispersal for a transgenic plant is very similar to that of its non-transgenic

counterpart. In the case of transgenic varieties, the main preoccupation is that transgenes might introgress into the wild relatives of the cultivated plants subject to biotechnology. In a recent review (1999), Ellstrand et al. presents clear and extensive evidence for the occurrence of spontaneous hybridization among most cultivated plants and their wild relatives. In some cases, hard-to-control weeds have evolved as the result of hybridization (Ellstrand 2001). Long-distance pollen dispersal occurs for only a small fraction of the pollen produced. However, the fact that a small amount of pollen travels considerable distances makes complete isolation almost impossible. Furthermore, the progeny of plants that have been pollinated unintentionally by transgenic varieties may serve as natural bridges to transfer transgenes to other non-transgenic cultivated varieties or to wild relatives (National Academy of Sciences 2002).

There is much less evidence on gene flow among cultivated varieties (Ellstrand 2001). However, high rates of crossbreeding among cultivated varieties of the same species are expected. In fact, there are several documented cases of transgenes flow into cultivated varieties for which the biotechnological developments were not intended. This is the case, for example, with canola (Hall *et al.* 2000). This and other examples point to the fact that the containment of transgenes within the cultivated populations for which they are originally approved will be practically impossible once transgenic varieties are commercialized or deregulated (Hodgson 2002), unless very effective methods are developed to arrest transgene flow.

Maize is an open pollinated and outbreeding species (Frankel and Galun 1977). Therefore, in Mexico, the various local varieties of maize, as well as the commercial hybrids, can crossbreed among themselves, just as they can crossbreed with maize native varieties, the teosinte species (see above). In cases where they are growing in proximity to local maize varieties or teosinte populations, transgenic varieties can be pollen donors for or acceptors from other maize varieties. In what follows we will summarize the evidence for gene flow from maize into teosinte populations in Mexico.

The closest wild relatives of maize are teosintes and these belong to several species within the same genus: *Zea* (Sánchez and Ruiz 1996). The geographical distribution of teosintes in Mexico is shown in Figure 1. Some teosinte species are also found in Central America and Nicaragua (Sánchez González and Ruiz Corral 1997, Serratos, in prep.). Some of the species or populations of teosintes may have become extinct due to introgression from cultivated maize (Small 1984). Most teosinte species can be hand pollinated with pollen from maize to yield fertile hybrids (Goodman 1995); although crosses with *Z. m. ssp. mexicana* are hard to achieve (Kermicle 1997) and hybrids from maize and *Z. perennis* are sterile (Doebley 1990). Maize can even crossbreed with species of *Tripsacum* (Goodman 1995), but this has not been documented under natural conditions. But in fields with abundant *Z. m. ssp. mexicana*, it is common to find spontaneous hybrids between it and maize (Wilkes 1977). Alloenzymatic analyses in samples of *Z. luxurians*, *Z. diploperennis*, and *Z. m. ssp. mexicana* suggest that low levels of introgression from maize to these teosintes have occurred (Doebley 1990). This conclusion has also been drawn from analyses of the internal transcribed spacer (ITS) sequence of ribosomal DNA (Buckler and Holtsford 1996). In contrast, cytogenetic analyses (Kato 1997) show that no introgression has occurred and this has been found also with alloenzymatic data from seed collections of teosintes that had been growing intermingled with maize (Doebley 1990). But this kind of

evidence has not been documented for *Z. m. ssp. parviglumis* because appropriate genetic markers were lacking.

The clearest evidence of introgression of maize alleles into the wild populations of teosintes obtained up to now, however, comes from the work of Blancas *et al.* (2001). This study used 18 alloenzymatic loci and analyzed sympatric and allopatric maize and teosinte populations. These authors found that population pairs of maize-teosinte had smaller genetic distances than any other population pair, including those consisting of two allopatric populations of the same species. They also analyzed individuals with morphological traits intermediate between maize and teosinte and concluded that these intermediates are hybrid individuals but represented a lineage that had evolved independently of the parental lineages.

Gene flow among maize native varieties and commercial hybrids in Mexico has been documented in various studies (e.g., Castillo González and Goodman 1997; Louette, 1997). In this case, the rates of outbreeding were high: up to 60 percent of the seed of a local race came from an improved hybrid that was growing in close proximity. This produced several local varieties that were very similar to improved hybrids (e.g., see references in Castillo González and Goodman 1997).

The above results suggest that it is very hard to maintain a maize variety genetically isolated from other nearby varieties. However, the production of certified seed has established that a distance of 200 m between cornfields is sufficient to achieve a high (but not absolute) degree of isolation. Data from the state of Nayarit, Mexico, suggest that at a distance of 200 m from an experimental pollen source, pollination in the receptor variety occurs, but when source and receptor are 300 m apart cross pollination is no longer detected (N. Ellstrand, personal communication). In India, on the other hand, 400 m is the minimum distance (Tunwar and Singh 1988), while FAO (1982) advises 600 m (Kelly and George 1998). A more efficient method to isolate different maize varieties might be to select for different reproductive phenologies (Kelly and George 1998). In any case, however, it is clear that in Mexico it will be very hard to isolate a transgenic variety from other varieties and from the teosinte populations in the areas where these grow (see Figure 1).

Gene movement in plants is not due just to the movement of pollen (gametes), but also to that of seeds and fruits (grains in corn), as well as movement of vegetative structures like rhizomes, bulbs, etc. In natural populations these movements depends on natural agents. However, in cultivated plants such as maize, gene movement depends, too, on the people in charge of cultivating or commercializing the plants. Although there are two important regulations covering seed exchange (The Federal Plant Varieties Law—Plant Breeders' Rights and the Federal Law on Seed Production, Inspection and Certification), agricultural practices that involve saving seed for future growing seasons and exchange of seeds among peasants without any regulation are very common in countries like Mexico. There, gene flow due to intentional or unintentional seed exchange plays an important role in determining population genetic structure and diversity patterns within and among varieties. These social flows are superimposed on the biological gene flows. Furthermore, most small peasants have several small fields dispersed in the landscape and peasants are unable of predicting and controlling gene flow among their fields and between these and fields of other peasants (Bellon and Brush 1994). This situation ensures that many populations are found one close to another one favoring gene flow among different varieties and races of

maize, as well as with teosintes that grow in the edges of cornfields or in nearby natural areas. Therefore, genetic diversity in Mexican peasant cornfields is very dynamic and land races are not static but are constantly evolving—maintaining the traits desired by farmers—from the influx of new genes and because of peasants' selection of preferred traits.

The entrance of transgenes into a population does not guarantee that these may persist. Introgression of transgenes in wild populations will readily occur if the hybrid's fitness is higher or equal to that of its wild relatives. Available data suggest that, in many cases, hybrid fitness is equal to or greater than that of wild individuals (Ellstrand *et al.* 1999), causing the evolution of new weeds (Ellstrand and Schierenbeck 2000). There is also some evidence for the extinction of wild relatives after hybridization with cultivated species (Ellstrand *et al.* 1999). Once introgression has occurred, the biological effect of transgenes will depend on the phenotypic effect that results from the expression of the inserted gene (Ellstrand and Hoffman 1990). The primary biological effects of some of the 22 transgenic varieties that have been approved and deregulated in the United States (see <www.agbios.com>) have been studied. The transgenes in these maize varieties confer herbicide resistance, resistance to various lepidopteran pests, male sterility, or a combination of these traits. In each case, the possibility of transgene persistence after gene flow and introgression into wild or cultivated populations and their biological effects merits an independent treatment.

In addition to the natural processes involved in determining the permanence of transgenes in natural wild or cultivated varieties, perhaps the most influential and least understood influence on genetic diversity and the “maintenance” of land races is farmer management practices, particularly the practices farmers use to choose seed for planting the following year. This fosters gene flow. However, transgenes may not be 'detected' by peasants unless they confer clear phenotypic traits that are amenable to selection. Some of these issues are discussed in Professor Altieri's paper.

1.2.2 Consequences of gene flow

Persistence of transgenes after gene flow

Transgene persistence depends for its effect on the plant's fitness relative to non-transgenic plants (equal to the product of survival and reproductive probability in comparison to plants not expressing the transgene). If the fitness of a transgenic variety is equal to that of a non-transgenic, then we say that the transgenes are neutral. For example, herbicide tolerance is probably a neutral trait in plants that are not treated with the herbicide for which transgenes were developed. But, for example, resistance to lepidoptera will increase fitness at sites where these insects normally would decrease the probability of survival and/or reproduction of plants. Finally, male sterility will decrease the probability of reproduction via pollen. It is clear that beneficial transgenes may persist in wild populations or local cultivated varieties after a single introduction. For this reason, it is important to maintain efficient monitoring schemes to evaluate native populations of teosinte and of local land races in Mexico. The possibility that these transgenes have already introgressed into land races in Mexico is still under debate, following the publication by Quist and Chapela (2001). We will describe this debate in the following section. Here, we will provide more details on the conditions that may mediate the persistence of transgenes once they arrive in the native population.

We might consider the case in which a single gene flow event occurs from individuals (donors) for which the transgene is fixed to a population of non-transgenic plants (cultivated and wild). Let us assume that in the progeny resulting from a hybridization event between these two populations, the proportion of the transgene is equal to p . Population genetic theory shows that, given a unique gene flow event, the transgene will remain at this frequency if it is neutral. But if the transgene causes an increased fitness in transgenic hybrids, the frequency will increase every generation until fixation in the receptor population is achieved. In contrast, if the hybrid individuals carrying the transgene have lower fitness than those that do not carry it, then the transgene frequency will decrease until it disappears (Ellstrand 2003). This is the simplest case. In the case that recurrent events of gene flow occur from a uniform source, transgene frequency will increase every generation—even in cases of neutral (but not detrimental) transgenes. In these recurrent gene flow events, advantageous transgenes would be fixed very rapidly, but even if the transgenes caused fitness decrement, they could eventually increase and maintain intermediate frequencies that will depend on the balance between negative selection pressure and gene flow rate (Ellstrand 2003).

Evolution of weeds resistant to herbicides or pests

Cultivated plants improved to resist pests by traditional breeding may hybridize with wild relatives, giving rise to new and more competitive weeds. In the case of transgenic plants that have been modified to be more resistant to pests not found in wild populations, the transgene will be neutral. In cases where transgenes confer resistance to herbicides, no fitness advantage would be expected if the herbicide is not used. Often, however, treated cultivated varieties and their wild relatives cohabit. In this case, the introduction of genes that make wild relatives resistant to herbicides may convert them into hard-to-control weeds as in the case of wild relatives or related local varieties (Hall *et al.* 2000). For example, canola volunteers may become hard to control. In canola there are now 4 HT types (Bromoxnyl tolerance was bred by Rhone-Poulenc Rorer, now part of Bayer Cropscience) and pursuit variety bred by mutagenesis. Plants resistant to the three herbicides were generated in order to try to make them different to wild relatives of canola. Although resistant wild plants can still be controlled by other herbicides, it is clear that once genes that confer herbicide resistance introgress into populations of wild relatives that are, in turn, in close proximity to the cultivated variety with the herbicide, hard-to-solve weed management problems can be created. If a sequential introduction of transgenes is followed in order to maintain the commercial value of the cultivated varieties, this would lead to use of different and/or potentially more potent herbicides that might be more harmful to the environment, although current research on herbicides is precisely aimed at developing less harmful compounds. To avoid this, it would be important to refrain from introducing more than one resistance gene in each variety or in varieties that can crossbreed, once they are released into the environment.

In the particular case of maize, the introduction of herbicide resistance would be problematic in sites in which teosinte is considered a weed that is controlled by the herbicide for which the transgene has been introduced. In this case, the introduction of the herbicide resistance transgene in teosinte would lead to the evolution of a more efficient weed that would have to be controlled with a different herbicide. A similar problem would

be generated in the case of transgenes that express the protein *Cry*. This protein is toxic for various insect pests, especially lepidoptera larvae (Sears *et al.* 2001). The best studied case concerns the deregulated varieties in the United States that have been genetically modified to express the protein *Cry1Ab* from a soil bacterium, *Bacillus thuringiensis*. These transgenic plants are also called *Bt*-plants and are said to express the *Bt* protein, which forms crystals that solubilize in the medial intestine of the European borer larvae of the lepidopteran, *Ostrinia nubilalis*. Once in solution, these toxins become active and adhere to the columnar cells of the intestine, forming ion channels and provoking epithelial cells to burst, causing the death of the insect larvae (Strizhov *et al.* 1996). Teosinte could become a more efficient weed if such resistance were introduced into teosinte populations that are naturally controlled by lepidoptera susceptible to this toxin. However, in this case, *Bt*-teosinte would be resistant to lepidoptera, but not to an adequate herbicide that would be needed for its control. However, we do not expect that hard-to-control weeds would evolve in either case from introgressed varieties of maize.

Male sterility

Male sterility should not cause detrimental biological effects in Mexico because it is very unlikely that these transgenes would reach Mexican varieties, and because the expression of this trait decreases fitness in both cultivated and wild maize populations with these genes.

Evolution of resistant insects and new pests

Under natural conditions, insect-resistant plants have evolved after millions of generations of long, coevolutionary history with their insect pests. The so-called coevolutionary 'arms-race' takes place in coexisting plants and animals under natural conditions. Both evolve resistance to each other as a result of their mutual selection pressures. Plants evolve resistance to their pests and this causes the evolution of new pests that are, in turn, resistant to the newly evolved plant types, and so on. These coevolutionary dynamics also occur between coexisting plant-pest species under agricultural conditions and it is expected to occur between transgenic varieties and the pests that these transformed plants resist. For example, the use of *Bt* plants can select and thus favor pests resistant to these toxic proteins. *B. thuringiensis* has been used as a microbial insecticide in organic agriculture for more than 30 years. As this toxin is degraded relatively quickly in the environment, insects are exposed to the toxic protein for limited time periods. Perhaps this is the reason that resistant pests have not evolved when *Bt* is used in this way. This fact has stimulated the use of this toxin in biotechnological developments of transgenic plants resistant to pests. However, in the case of transgenic plants that express the *Bt* toxin, the evolution of *Bt*-resistant pests is more plausible, if resistance management programs are inadequate. This can happen because of the extensive and intensive use of transgenics that express *Bt*, and hence pests would be exposed to higher concentrations of this toxin and for periods of time longer than under organic agriculture. The evolution of resistant pests would make transgenic *Bt* plants and the biological use of this toxin useless (Gould 1998).

To avoid evolution of resistant pests among insects exposed to transgenic *Bt* plants, various methods have been developed (Andow 2002). Among these are the use of refuges in which not resistant and non-transgenic plants are cultivated, the use of different variants of the toxin to avoid fixation of a resistance mechanism against a particular toxin variant, and avoid introducing different resistance transgenes into the same varieties to impede that

evolution of resistance to multiple toxins occurs. Another common practice is rotation among resistant and non-resistant plant varieties to avoid a directional and consistent selection pressure on insects to evolve resistance. Therefore, it is clear that unintentional and uncontrolled gene flow of pest-resistant transgenes into areas in which the above methods are not used would lead to the evolution of resistant pests that become hard to control using *Bt* and a different insecticide should then be introduced. It is important to emphasize that the success of using *Bt*-plants depends on an integrated pest management system that is unlikely to work in most areas of Mexico.

Genetic erosion

Gene flow of new alleles causes an increase in local genetic diversity at least temporally. The circumstances under which the introduction of a new allele can lower genetic diversity are very restricted. But this can certainly occur if the newly introduced allele confers a fitness benefit that leads to its fixation and the loss of wild-type alleles within the same locus. In rare cases, closely linked loci to the fixed new allele can also be fixed and alternative alleles lost. This phenomenon, by which alleles in closely linked loci are fixed when an allele of the neighboring loci confers very high fitness, is known as “hitchhiking selection.” However, in open-pollinated species like maize, recombination will guarantee that this phenomenon rarely occurs and it will affect very small portions of the genome (Ellstrand 2003).

Unexpected effects

Offspring of transgenic plants that have unintentionally pollinated plants for which transgenes were not intended may have unexpected effects in other organisms that were not the target of the biotechnological development. For example, the expression of toxins may harm insects that are beneficial to the ecosystem. The possible detrimental effect of the *Bt* toxin on monarch butterflies has been extensively investigated. Losey *et al.* (1999) showed that pollen from *Bt* transgenic plants that is deposited over leaves of *Asclepias* plant species is toxic for monarch butterflies larvae that feed on it. These first experiments were performed under laboratory conditions and the same authors recognized the need for field studies. Several other laboratory and field studies followed (Hellmich *et al.* 2001; Oberhauser *et al.* 2001; Sears *et al.* 2001; Stanley-Horn *et al.* 2001; Zangeri *et al.* 2001) resulting in some controversy. While some studies found significant effects on larvae survival exposed to *Bt* pollen in comparison with unexposed ones, others did not (Wraight *et al.* 2000). But it is clear that the effect depends on the concentration and the specific variety of *Bt* tested and it seems clear that very small effects are expected, given the concentrations found under field conditions. Nonetheless, it is advisable to develop transgenic plants that do not express the *Bt* toxin in pollen grains, but only in the plant organs attacked by the pests.

Even though the documented effects of *Bt* on the survival of monarch butterflies are negligible, it is clear that ecosystemic effects of the expression of transgenes are possible. Therefore, it is important to undertake field research and long-term monitoring on possible unexpected effects of transgenes released into the environment. For example, although *Cry* proteins are naturally produced by soil bacteria, those from transgenic plants might be quantitatively and qualitatively different to the ones from natural soil bacteria, and because it is now known that *Cry* proteins from transgenic plants persist in the soil for much longer

time periods than was suspected before, their long-term effects on soil flora and fauna should be investigated (Stotzky 2002).

In addition to effects on non-target organisms, a transgene may have additional effects on the carrier plant itself, depending on the environment in which it is grown. These collateral effects are the so-called pleiotropic effects and are common to wild-type genes and transgenes alike. While transgenic varieties are being developed in research laboratories or industries, different lines are carefully tested for avoiding unexpected effects. These explorations generally reveal that only a small fraction of the lines fulfill the required conditions of expression, stability and predictability, once the lines are exposed to various conditions. But the tested environments are generally limited, in comparison to those experienced by plants once they are released into the environment. The difficulty of finding an adequate line is determined, in part, by the fact that the expression of a transgene is affected by the genomic site in which it is inserted. This site cannot be determined, controlled *a priori*, or fixed for plants and animals with the transformation techniques available now. For example, recombination could cause alterations in the expression of transgenes in subsequent generations once a transgenic plant is released into the environment and left to outcross. Some genomic sites might be more prone than others to such instabilities (see section below on the instability of transgenic genomes). In conclusion, it is mandatory to maintain systematic monitoring schemes over the whole range of environments in which transgenic varieties are released.

An interesting example of an unexpected effect has been reported for *Bt* lines in maize. Saxena and Stotzky (2001) compared different *Bt* maize transgenic lines and found that they had significantly higher (33–97 percent) lignin content, compared to corresponding isogenic wild type plants under growth chamber and field conditions. Furthermore, lignin content was always significantly higher in field-grown plants compared to that of plants grown in laboratory growth chambers. In analyzing the potential effects of these differences in the environments, the researchers concluded that some might be beneficial, such as the greater strength of stems with higher lignin contents, or the possible positive effect of lignin on soil organic matter and increased control of soil erosion. However, the authors argued that other consequences might be detrimental, such as the prolonged accumulation of the toxin in the soil and the difficulty of digesting transgenic plants with higher lignin contents when maize was used for animal feed. In any case, the key issue here is that unexpected effects due to pleiotropy may indeed occur. Also, given our knowledge about plant physiology and development, it is still very hard to predict all the possible outcomes of genetic transformation with foreign sequences. In the particular case discussed here, we still do not understand the genetic mechanism that links *CryIAb* metabolism with lignin synthesis and probably with other processes that have not yet been explored.

2 State of Knowledge: Areas of Debate

In this section, we discuss the most important debates for each of the sections presented above.

2.1 Mexico as center of origin of maize diversity: Present state of the existent biodiversity of local races and wild teosintes

There is still debate on the number and genealogy of maize varieties and on some aspects of the evolutionary history of cultivated maize in relation to teosintes. Different authors draw different distinctions between maize varieties, based on different criteria, and it is important to clarify this situation in order to enable objective evaluations of the impacts of transgenes in maize biodiversity in Mexico. At the end of this essay we will provide some specific recommendations on this subject.

2.2 Environmental effects due to the release of transgenic maize varieties

2.2.1 Gene flow

Discussions about the environmental effects of transgenic maize varieties center around the possibility of gene flow from cultivated maize to its wild relatives populations. However, gene flow among maize cultivated varieties is equally important. Despite the fact that in Mexico there is a *de facto* moratorium for semi-commercial and commercial cultivation of transgenic maize since 1998, recent reports indicate that transgenes from several deregulated lines in the United States might have already introgressed into local land races of Oaxaca, Mexico. But this is still under debate and it is important to unequivocally prove it.

Presence of transgenes in Mexican maize land races

The study published by Quist and Chapela (2001) was the first report based on empirical evidence citing the presence of transgenes in Mexican maize land races. These authors found polymerase chain reaction (PCR) evidence for the presence of the 35S promoter sequence from the cauliflower mosaic virus in genomic DNA of Mexican maize land races. This promoter has been extensively used in transgenic plants deregulated up to now. The authors also found the 3' end NOS terminator from *Agrobacterium tumefaciens* and the *CryIAb* gene that codifies for the insecticide protein *Bt* from *Bacillus thuringiensis*. However, a note was published shortly after the publication from Quist and Chapela (Hodgson 2002) that questioned Chapela's findings. The main arguments of this note were based on a study published on the Internet page of the International Maize and Wheat Improvement Center (CIMMYT, see also Christou 2002) where analyses of samples of Oaxacan maize collected between 1999 and 2001 were shown with negative PCR results for the same 35S sequence. A few months after Quist and Chapela publication in *Nature*, a letter from the editor of the journal (Editorial Note 2002) stated that *Nature* could not attest to the veracity of the authors' conclusions because of technical problems that had been summarized in two other letters written by several scientists (Metz and Fütterer 2002; Kaplinsky *et al.* 2002). These scientists argued that PCR-based techniques might be prone to experimental artifacts, particularly when two rounds of PCR were used to obtain positive bands. They also argued that it is necessary to have southern hybridization experiments to be able to reassure the presence of transgenic sequences within the genomes of maize land races. Finally, they also pointed out that the results derived from the 'inverse-PCR' contain artifacts due to the presence in the maize genome of sequences closely similar to some stretches of the 35S promoter sequence. Based on this, they argued that the evidence for transgene instability lacked validity. However, both groups of scientists concluded that it

was quite probable that transgenes were already present in Mexican maize land races—although it was premature to draw conclusions based on Quist and Chapela’s results. In the same issue, Quist and Chapela (2002) published a letter containing new data from dot-blot hybridization analysis of a few samples that confirmed their conclusions of the presence of transgenes in the genomes of local land races and accepted that some, but not all, of the results derived from ‘i-PCR’ analyses were artifacts. However, their work reaffirmed the researchers’ main conclusion that transgenes are found in Mexican maize land races.

Motivated by the studies of Quist and Chapela, the National Institute of Ecology (INE) and Conabio are coordinating experimental work at two laboratories (Dr. Rafael Rivera at Cinvestav, Irapuato, and the author at UNAM, Mexico City) to test if transgenes have indeed introgressed into Mexican varieties of maize land races. The first results of these studies have been submitted to *Nature* but the study was rejected on the basis of technical criticisms. Some of these are valuable and are being considered, and additional experiments are underway before the paper is resubmitted (see Appendix 1). Nonetheless, results obtained in these two laboratories suggest that transgenes are indeed found in some of the maize land races collected in the states of Oaxaca and Puebla. It is of first priority to resolve unequivocally if transgenes have indeed introgressed into Mexican maize land races. Some well-documented consequences of introgression have been discussed in previous sections. Consequences for which there is still debate or a lack of knowledge are discussed in the following sections.

2.2.2 Consequences of gene flow

Ecosystemic effects on unrelated species and non-target species

Transgenic varieties may impact biodiversity by affecting the environment and other species not related to the transgenic species or that are not the target of the biotechnological development. Such impacts occur at the ecosystemic level. There are still few data to substantiating such effects. Among the impacts on non-target species are those that decrease the survival or reproduction of beneficial insects such as pollinators or pest parasites. The best-studied case is the effect of *Bt* maize on monarch butterflies that was mentioned above. Although data from investigations of this have concluded that effects are marginal, it is important to continue exploring such effects with long-term studies for other cases.

3 Priority Themes in Which More Research Is Needed to Improve Understanding on the Risks of Biodiversity

Areas for which research is most urgently needed are:

3.1 Existing biodiversity of local races and wild teosintes

Criteria to distinguish among maize races must be updated using molecular markers that enable genealogical analyses to be performed. These analyses will establish genetic relatedness among races, and the criteria used by peasants to distinguish among races may be mapped into such genealogical analyses. These markers could be useful, allowing us to produce standardized criteria for characterizing maize biodiversity as well as delineating the relationship between cultivated races and wild relatives. Efforts are being accomplished towards this end, but more extensive, detailed sampling is needed. This will produce a

fundamental reference to evaluate the impact of biotechnology on maize biodiversity. In Appendix II we detail some priority actions that may also contribute to avoid extinction of local maize races and their wild relatives.

3.2 Environmental effects due to the release of transgenic maize varieties

3.2.1 Gene flow

Presence of transgenes in Mexican maize land races

It is of highest priority to verify the findings of Quist and Chapela (2001). If these results are corroborated, it will be necessary to establish stringent means to avoid further gene flow into local races and wild relatives. It is also important to investigate whether there has already been gene flow into teosintes and if the germplasm banks are free of transgenic material. If gene flow has already occurred into local races, it will be important to study which races have been introgressed, the spatio-temporal dynamics of the transgenes in different agricultural conditions, the pleiotropic effects of the transgenes and their genomic stability. Also, it will be important to verify the source of transgenes found up to now. One possible source is the rural stores that receive part of the maize grain imported from the United States. Some of this grain is unlabeled transgenic grain of the deregulated varieties available in the United States. All aspects discussed in the previous and the following sections should be investigated in case the introgression of transgenes into Mexican maize varieties is confirmed.

3.2.2 Consequences of gene flow

Horizontal gene transfer: Antibiotic resistance

The possibility that any of the DNA sequences inserted into transgenic plants can be transferred to unrelated species via horizontal gene transfer (especially by means of bacteria or viruses) is being strongly debated. This has occurred naturally in evolutionary history but the key question is whether the novel sequences created by DNA recombination pose special ecological risks. Consequences of horizontal gene transfer are hard to predict, but some could be important. Such would be the case of the transfer of genes conferring antibiotic resistance into pathogen microorganisms that might thus become resistant to selected antibiotics. Many deregulated transgenic lines carry such antibiotic resistance genes (see Appendix III).

Naked DNA accumulation in the environment: Biotoxic waste?

Some researchers are worried about the accumulation of “naked DNA” (PCR-amplified sequences, DNA sequence chimeras that are not found in nature, plasmids and other vectors) that might remain as waste from the biotechnical industry or from DNA accumulation after the death of transgenic plants released into the environment (in our case, transgenic maize). These organisms could leave behind large quantities of naked recombinant DNA in the environment after they die and decay because there is evidence that nucleic acids may retain biological activity for long time periods (Lorenz and Wackernagel 1994; Nielsen *et al.* 1998). Can these nucleic acids be biotoxic? What will be their ecological effect in the short and long term? These questions are closely related to

those of the previous section because some of these nucleic acids that may be new to nature because of their quality and quantity might then become incorporated into bacteria and viruses via horizontal gene transfer. It is urgent to investigate these issues.

Instability of transgenic genomes

There are many unresolved questions concerning the structure and dynamics of genomes. Many aspects of transgene integration in higher plants and animals are not understood and cannot be controlled during genetic transformation. A deeper knowledge of genome structure and dynamics would enable better predictions about transgene stability and, hence, about its ecological and evolutionary effects once transgenic animals or plants are released into the environment. It would be important to be able to predict the site of transgene insertion and predict if there are genomic positions that are more suitable and stable than others. Since it is currently not possible to predict the insertion site of transgenes in higher plants and animals, this is the reason why transgenic lines are selected empirically *a posteriori* using laboratory and field experiments. For example, some of the sequences used in most transgenic lines may become sites with high recombination rates. Much more research is needed on this and related issues in maize and other released transgenic plants (see Appendix IV). But research and monitoring programs that address these problems rely on accurate information on all the plasmids and transgenic sequences used to generate each organism that is finally released into the environment and deregulated. It is crucial, therefore, that for companies or any other institution that generates transgenic varieties to make readily available information on the transgenic construct, its chromosomal map location as well as at least five kilobases around the original insertion site. For maize varieties, this information could be precisely referenced with markers that are available for the whole genome. This information would aid the long-term monitoring programs that are needed if we want to evaluate the stability of transgenes once they are released into the environment. In conclusion, companies that produce and commercialize transgenes generally should provide the scientific community and the general public the information and materials that are necessary to undertake this type of monitoring.

Plausible impacts of new biotechnological developments in maize that are not yet deregulated and that produce pharmaceuticals and industrial substances

Given that gene flow and introgression from transgenic varieties into local maize races that are used for human and/or animal food are clearly possible, if not yet a reality (see discussion on Quist and Chapela, above), it is particularly worrisome that introgression from transgenic plants that produce toxic pharmaceuticals or industrial biochemicals may occur (Ellstrand 2001). This could affect food security in Mexico and in the whole world and it is of the utmost urgency to evaluate this possibility and, if necessary, to stop these types of developments in open pollinated plants that are used for human and animal consumption, such as maize. We summarize here the risks that the newest developments in maize imply.

The Information Systems for Biotechnology web site and database of United States' field tests of transgenic plants provides a list of 1065 field tests approved since 2001. Each trial implies field tests and most of them are for maize (more than 700) with phenotypes similar to the deregulated maize varieties that have been considered in the previous sections of this

essay (alterations to achieve herbicide resistance, insect resistance, male sterility or a combination of these). However, 216 other field trials imply other alterations, such as changes to the profile of the amino acid or oil content, or yielding higher protein production, higher yields, or altered morphology or phenology. Very limited information on these varieties is available because they are not yet deregulated and hence it is hard to evaluate their possible biological effect (National Academy of Sciences 2002).

It is, nonetheless, extremely relevant to note that 37 of the entries for maize since January 2001 correspond to varieties that are characterized as “pharmaceutical proteins,” “novel proteins,” or “industrial enzymes.” This number of new developments in maize is increasing at an accelerating rate. It is noteworthy that for all other plant species, there are only nine entries with these types of transgenes. Therefore, it is clear that biotechnological companies have selected maize as a “bioreactor” for the production of chemicals of novel nature and application. What will be the effect of these new compounds once they are released into the environment? Probably most will be innocuous to consume, or will not have devastating consequences, but we should worry that so many new products of pharmaceutical or industrial application are being developed precisely in maize. In the event that any of these products is toxic to humans or animals and its pollen should happen to fertilize varieties generally used for human and/or animal consumption, we could expect negative consequences of substantial concern. In such a case, one single contamination event, even before introgression, would be problematic if the toxin concentration level reached in the hybrid seeds and the variety’s hybridization rates were high enough.

The present laws regulating transgenic varieties in the United States would not suffice to control escapes from the trial fields where the non-deregulated varieties were being grown (APHIS 1997; National Academy of Sciences 2002). Even a few such escapes to fields of non-transgenic maize could have devastating consequences once the new varieties introgress and multiply within the United States and abroad. Given that, at present, it is not easy to identify corn grains and plants from transgenic lines that produce these types of industrial chemicals, it would be very hard to ascertain if these transgenic varieties arrived among the local maize races in Mexico. If the conclusions advanced by Quist and Chapela should be confirmed, they will show us that it is very hard to prove to the highest scientific standards the presence of transgenes in local maize races—even for the transgenic varieties that have been deregulated and for which information might be available to allow monitoring. By contrast, monitoring for transgenes of as-yet non-deregulated varieties, and for which very little information is available, will be substantially harder. More readily available information on new developments is therefore urgently needed.

The information summarized in this essay suggests that unless pollen and seed of these new developments is completely contained, it will be very probable that their transgenes will reach Mexican local races of maize. This would probably occur even if Mexico’s moratorium for transgenic maize were continued. If genes that express industrial biochemicals reach the teosinte populations, then it would be impossible to eliminate them unless they happened to confer clear fitness disadvantages in hybrid plants. Therefore, contamination of local maize races in Mexico by genes that codify for toxic substances is probably the issue of highest preoccupation linked to the question of gene flow from transgenic maize varieties and its consequences to biodiversity, but also to human and/or animal health. We consider it an urgent problem of the highest priority (see Appendix V).

Appendix I: Studies to Document Introgression from Transgenic Varieties into Local Maize Races in Mexico

After the publication by Quist and Chapela (2001), INE and Conabio in Mexico asked two Mexican laboratories from public universities to undertake detailed studies to corroborate or refute the study published in *Nature* concerning the possible introgression of transgenes into local maize races of Oaxaca and Puebla. Maize collections were made in these two states and data from PCR, BASTA resistance, *Bt* protein activity and Southern-blot hybridization experiments were collected. The results obtained up to now indicate that transgenes are present in the genomes of Mexican maize local races collected. This study is being completed and results are expected in a few months. Other members of Cinvestav are also performing molecular analyses to test for the presence of transgenes in local maize races for samples collected in other parts of Mexico. Results from these studies should also be ready by January 2003.

Appendix II: Strategies to Prevent Extinction of Maize Varieties and of Teosinte Species

- Start a program for revising collections and seed banks as well as improving the infrastructure of public seed banks and herbaria. This should be done for both maize and teosinte. New collections should consider population variability and spatio-temporal structure of maize varieties and teosinte populations. This information will be important in considering different causes for extinction of each race. For example, land races that grow in close proximity to improved hybrid maize varieties are more prone to be locally substituted. Also, social aspects, such as migration, should be considered while evaluating the local extinction probability of each race. For example, in many rural areas of Mexico, most young peasants have left the land and only older peasants remain and still cultivate maize. Finally, collections of each race should be constantly updated because maize races are dynamic units that are under constant natural and artificial forces of selection, and constitute different sources of gene flow that avoid inbreeding.
- A fundamental issue is to test the maize seed banks to see if they are free of transgenes. Transgene-free material might be very important for introduction and regeneration programs of local races that become extinct or introgressed by transgenes. An updated methodology should be established to guarantee that international and national maize seed banks in Mexico are free of transgenes.
- Molecular markers should be set up to establish the genealogical relationships among maize races and between these and teosintes. These analyses should be superimposed on the morphological and social traits considered in the establishment, distinction and classification of maize races.
- Promote a net of maize seed banks in different regions of Mexico with trained personnel to control the access and use of the collections and the protected areas nearby, where local races or teosintes are conserved. Such a system would provide input to *in situ* and *ex situ* conservation programs.
- Establish a priority list that includes those maize races or teosinte species that are more prone to extinction. Also, create economic incentives to favor the *in situ* and active conservation of local varieties that are free of transgenes. This would require validation of practical and cheap detection techniques.
- Start a nationwide system to monitor the entrance and spatio-temporal dynamics of maize transgenes into Mexico.
- All teosinte species should be incorporated in CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora). Promote natural reserves for the *in situ* conservation of teosinte species. The only natural reserve now is in the Sierra de Manantlán, Jalisco, for *Zea diploperennis*.

Appendix III: Antibiotic Resistance in Transgenic Plants

The origin of antibiotic-resistant bacteria is not a new problem, but the generalized use of antibiotics is creating new problems (Kruse and Sørum 1994). Transgenic constructs that are inserted into plant genomes generally contain genes that make plants resistant to a specific antibiotic. This gene is used as a selection marker during the development of the transgenic variety to select those individuals and/or cells that have incorporated the construct from those that have not, when they are grown in media with the selected antibiotic. These markers remain in plant genomes and are expressed once plants are released into the environment, although they are not necessary for the expression of the gene that is the object of each transgenic variety. Many of the deregulated varieties that have been released into the environment up to now carry antibiotic resistant genes. New developments are using techniques that excise the resistance genes before the line is approved for release. This is achieved by inserting the resistance gene between two homologous DNA stretches that pair with each other. During meiosis, at recombination, the DNA fragment between these homologous DNA stretches is excised because the flanking regions complement each other (Meyer 2000). Another means of mitigating the possible risks derived from the expression of antibiotic-resistant genes in transgenic varieties is to reduce the spectrum of markers used. This reduces the chance of generating genotypes with resistance to multiple antibiotics.

Appendix IV: Horizontal Gene Transfer and Stability of Transgenic Insertions in Transformed Plants

Traditional breeding and hybrid selection in plants and animals generally starts with artificial fertilization and, hence, the combination of whole genomes that in nature could or could not breed. This implies qualitatively different risks from genetic transformation using recombinant DNA technology. The latter may insert stretches of artificially assembled DNA sequences from different organisms (viruses, bacteria, other plants, animals) into the genomes of plants or other organisms. The organisms that provide the transformed sequences may have a completely different evolutionary history from that of the receptor organism and, hence, this technology transcends the limits of reproductive barriers that have been established after eons of evolution. Therefore, it is important to provide scientific evaluations of the consequences of such biotechnological plant (microorganism and animal) genetic transformations in, for example, the likelihood of horizontal gene transfers, or the origin of new viruses that could become pathogenic (see examples in Ho *et al.* 1999; 2000a; 2000b). There is still debate around this and related issues, and more research is needed.

Related to these issues is, for example, the question of whether the use of DNA sequences containing zones that experience high rates of recombination affect fitness of transgenic constructs and/or of the organisms that carry them? It is suspected that the CMV 35S promoter that is used in most transgenics developed up to now contains sequences that promote high recombination rates, altering the recombination probability of the transgenes associated with this sequence. This has been studied in rice and it renders the segregation ratios and the evolutionary dynamics of transgenes hard to predict (Kohli *et al.* 1999). Another type of recombination studied by the same author occurred among plasmids, when several plasmids were introduced into plants by bio-ballistic techniques. In the course of the transformation, certain genetic sequences of the plasmids were observed to recombine via the 35S promoter. This occurred both by micro-homological mechanisms and by illegitimate recombination. Other authors (Srivastava *et al.* 1996) have observed that transgenic sequences are eliminated after several breeding generations. This could be due to structural instabilities caused by recombinations among multiple copies of transgenes. It is important to check if the loss of transgenes caused by this type of recombination also causes the loss of some genes or gene fractions endogenous to the plant. Another question for research concerns the effect on plant fitness of a genetic transformation inserted in different genomic contexts. For example, can the level or site of transgene expression be affected by proximal regulatory or enhancer sequences, or by chromatin structure at the site of insertion?

Appendix V: Risks Associated with the Generation of Transgenic Plants that Produce Pharmaceuticals or Industrial Substances

New biotechnological developments in plants are geared to produce pharmaceuticals and industrial biochemicals. Many of these developments concern plants that are commonly used for animal and/or human consumption. For example, in the United States, there are several applications for this type of development. We must anticipate the possible effects of any chemical residue from these transgenic plants on biodiversity, soil and water quality, especially in those cases where these chemicals do not degrade rapidly or may be prone to bioaccumulation. Terrible consequences may be foreseen in those cases in which the chemicals or pharmaceuticals enter the trophic network, once pollen from the transgenic plants that are not suitable for consumption (human and/or animal) reach varieties of the same plant species (or their wild relatives) that are used for food, or if seeds from the industrial transgenic plants are mixed with those of non-transgenic varieties. The famous case of the StarLink maize is an example of how, despite regulation, a product that was approved only for animal consumption reached the human food chain. This happened in the United States, where regulation laws are clear and the mechanisms to enforce them exist. In Mexico, the regulatory standards and laws concerning transgenic plants are still being revised and their enforcement implies major challenges that will have to deal with the unregulated use, distribution, exchange and storage of seeds, the great diversity of ecological and socioeconomic conditions of agriculture in the country, and the great diversity of varieties of cultivated plants and their wild relatives. It is, therefore, urgent to discuss the type of risks involved in the various developments of industrial transgenic plants, especially maize, even before they are deregulated in the United States.

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