Maize and Biodiversity: The Effects of Transgenic Maize in Mexico

Chapter 3
Assessment of Effects on Genetic Diversity

for the Article 13 Initative on
Maize and Biodiversity

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Abstract

Phenotypic diversity among maize landraces is obvious in kernel color, ear and kernel shape and size, and agronomic traits such as plant height and length of the growing cycle. Traditionally, this phenotypic diversity has been used to classify populations into "races." Currently, 59 races of maize have been described in Mexico. According to DNA analyses, these races are organized as a continuum and their differentiation is mainly due to isolation by distance. Recent genetic studies of landraces in Oaxaca (Mexico) have shown that gene flow between maize populations is quantitatively important but that management of seed by farmers maintains strong agromorphological differentiation between maize populations.

In traditional agricultural systems, farmers play a wide range of roles with regard to seed production and maintenance. They conserve the genetic resources; they select and plant seed from their varieties; and, after harvest, they are the main consumers of their products. In Mexico, the traditional system coexists with the modern agricultural sector but the subsistence-oriented system is much more widely distributed. In this system, landraces are the result of continued evolution influenced by various factors: 1) *Seed recycling:* Saving seed from one season to the next is an almost universal practice among small-scale Mexican farmers. At least from the farmer's perspective, seed selection may also be fundamental to maintaining the integrity of a landrace, which can be lost easily through hybridization; and 2) *Seed flows:* Mexican farmers commonly acquire seed from other farmers or sources within or outside the community for several reasons, including experimentation, starting to farm, and lack of sufficient seeds. Thus, traditional farmers actively maintain landraces as dynamic entities.

Farmers are willing to modify introduced maize cultivars through recurrent hybridization with the local genetic material (process of creolization) to improve their local performance and consumer acceptability. They do not consider this process as "contamination." However, if the introduction of modern varieties becomes a permanent and pervasive process, a threshold could be reached above which gene swamping from those cultivars would reduce or eliminate the genetic diversity of local landraces. Furthermore, the evolution of landraces, which is based on hybridization, recombination, and selection, may follow paths unsuspected for modern varieties. Through recombination, genes belonging to a specific variety can migrate into new genetic backgrounds where new linkages and gene interactions may modify the expression of transgenes in an unpredictable fashion.

Teosinte, the closest wild relative of maize, is considered a weed in Mexico and farmers attempt to control it in their fields. Co-occurrence of maize and teosinte exists in several places in Mexico (Balsas, Chalco, the Central Plateau). Teosinte usually flowers two to three weeks later than maize but overlap in flowering times may occur. Furthermore, there are genetic systems that limit but do not completely exclude crossing between maize and teosinte. Evidence of the extent of introgression of maize alleles into teosinte is mixed. The most convincing example of introgression is provided by *Z. diploperennis*. In contrast, Kato (1984) was not able to find genetic evidence for introgression. The presence in the fields of plants that appear to be F_1 hybrids between maize and teosinte is well documented, but information is lacking about the behavior of the advanced hybrid generations. It also remains to be determined whether

hybridization between different genomes results in genome instability and gene movement within the maize genome.

Different agroecosystems are likely to present very different selection pressures. Thus, any beneficial effects of a transgene, for example, in an industrial agricultural setting cannot be assumed to exist in a traditional agriculture setting or among wild-growing relatives such as teosinte. Major pests of maize in Mexico are Lepidoptera, which may be susceptible to most of the Bt varieties that have been commercialized in the United States. However, further information is needed on how limiting these pests are in traditional maize agriculture and in teosinte. If herbicide tolerance genes are introduced into other genotypes, these in turn may become herbicide tolerant. This could be of benefit for local farmers, if they can or are willing to use the appropriate herbicides, and if the patent owner tolerates this inadvertent escape without suing the farmers. If herbicide tolerance is introduced through gene flow into teosinte, a potential tool for control of teosinte in maize fields may be lost.

Whether or not a transgene will spread into landraces or wild populations depends on a number of factors, including the level of gene flow in any given growing season and in successive seasons, and the selective effect of the transgene. If transgenes are selectively favored, selection may lead to a reduction of genetic diversity in the genomic vicinity of the gene in question. The size of the affected region subject to reduction in genetic diversity ("genomic window") is proportionate to the selective advantage of the gene under selection and inversely proportionate to the level of recombination. In maize, which has high rates of recombination, the region showing reduced diversity as a result of selection on a single gene would be small (one hundredth of one per cent). Thus, any effects of a single transgene on the genetic diversity of a landrace or of teosinte are likely to be insignificant from a biological point of view, unless there are high levels of gene flow from transgenic cultivars to landraces and teosinte populations, in which case the native genetic diversity may be displaced by the limited diversity of the transgenic cultivars.

The consensus at this stage is that transgenic sequences are present in Mexican maize landraces in the field although not in the CIMMYT gene bank. However, this leaves many open questions, including the geographic magnitude of the transgenes (how widespread are they?), their local intensity (what is their local frequency?), the identity of the transgenes (are they only from commercialized varieties, such as those conferring Bt and herbicide resistance, or are they from as yet uncommercialized genotypes, such as maize transformed for pharmaceutical production?), their possible presence in teosinte, their source(s) (local government stores, emigrants to the United States, seed companies, or other origins), the fate of transgenes in landraces and teosinte, and the role of farmers and others in gene flow by pollen and seed, involving transgenic sequences.

The processes that generate and maintain the genetic diversity of maize are very dynamic and preservation of diversity should be based on the preservation of these processes as well as the conservation of landraces, per se. We contend that establishing more effective selection schemes on the part of farmers may constitute an effective incentive for farmers to maintain their landraces. Farmers can actually be trained to conduct some degree of plant breeding, if they do not already do so. This type of outreach would require an active extension service, a network of NGOs or local cooperatives, or a network of technical schools. Preservation of genetic diversity

can be achieved through more active use of the diverse landraces and their diverse products, especially in urban areas where many of the maize products are currently very standardized and uniform. Education of urban consumers about diversity of maize and its products would help promote the use of products from these landraces, permitting the conservation of the diversity in the fields. Additional research is needed in the areas of flowering biology, gene flow, and reproductive isolation of maize. Furthermore, studies that document the extent of the distribution of transgenes among maize landraces and teosinte populations need to be conducted and published in peer-reviewed journals, following protocols that rely on replications and blind tests under the supervision of outside advisors.

Introduction

A loss of genetic diversity of domesticated and wild relatives is cited among the potential drawbacks of the introduction of transgenic crops. Whether this actually occurs depends on various biological and human parameters. In this chapter we present these parameters and describe their effects in the particular case of maize in Mexico. An effect on genetic diversity, if any, will require two essential conditions. Firstly, gene flow has to take place between transgenic and non-transgenic populations. Gene flow is defined here as the transfer of genes from one population or locality to another. Gene flow can take place either via pollen or seeds.[1] For example, seeds of a transgenic variety can be planted in a field mixed with seeds of non-transgenic varieties. Alternatively, fields of transgenic and non-transgenic (presumably traditional varieties) are planted within close proximity (several meters to 100s of meters) of each other. Hybridization between these plants (i.e. crossing by pollen transfer) can lead to hybrid seeds, which, if they are part of the seed stock saved by the farmer, can engage in another hybridization cycle in the next season, and so on. Secondly, gene flow has to be of such magnitude as to displace the native diversity. Figure 1 presents a sketch of a local system in which gene flow may take place between a transgenic and a traditional crop. It shows also the parameters that influence the direction and magnitude of this gene flow. In this chapter, we discuss several of these parameters as they may affect gene flow in Mexican maize, the potential role of transgenes in affecting gene flow and its effect on genetic diversity, the available data on the presence of transgene in Mexican maize landraces, and possible solutions to maintaining maize landrace diversity.
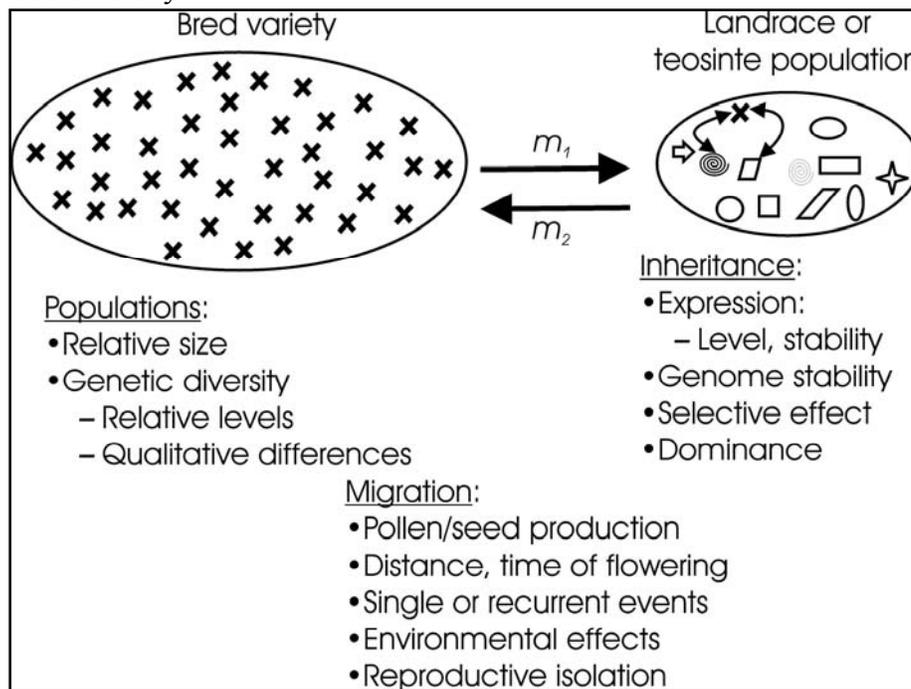
Figure 1. Factors influencing gene flow and potential genetic assimilation by gene flow.

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[1] Horizontal gene flow between genomes of different organisms takes place on an evolutionary time scale and is, therefore, probably too infrequent to be of consequence from the standpoint of this discussion.

1-Description of land races of maize and of species and subspecies of teosinte.

The current taxonomical situation for maize and teosinte is summarized from Doebley (1990) in Table 1. It is worth noting that *Zea diploperennis* was discovered by Guzmán in 1978 and described by Iltis et al. in 1979. A new species from Nicaragua and belonging to the section Luxuriantes has been described recently (Iltis and Benz, 2000). These discoveries illustrate that perhaps not all the diversity and taxa of teosinte are fully known yet.

Table 1. *Zea* taxonomy of Doebley (1990) compared to that of Wilkes (1967)

Wilkes (1967)	Iltis and Doebley (1980), Doebley (1990)
Section *Euchlaena*	Section *Luxuriantes*
-	*Zea diploperennis* Ilitis, Doebley & Guzmán
Zea perennis (Hitchc.) Reeves & Mangelsdorf	*Zea perennis* (Hitchc.) Reeves & Mangelsdorf
Zea mexicana (Schrader) Kuntze	
Race Guatemala	*Zea luxurians* (Durieu & Ascherson) Bird
	Section *Zea*
	Zea mays L.
	subsp. *mexicana* (Schrader) Iltis
Race Chalco	Race Chalco
Race Central Plateau	Race Central Plateau
Race Nobogame	Race Nobogame
Race Balsas	subsp. *parviglumis* Iltis & Doebley
Race Huehuetenango	subsp. *huehuetenangensis* (Iltis & Doebley)
Section *Zea*	
Zea mays L.	subsp. *mays*

Note: *Zea mays* L. subsp. *mays* is the taxon corresponding to the cultivated maize. All other taxa are teosinte. Teosinte taxa closest to maize are annual and belong to the section *Zea*.

Maize landraces

There is a great diversity of landraces in Mexico (Sánchez et al. 2000a,b). Phenotypic diversity is obvious in kernel color, ear and kernel shape and size, and agronomic traits such as plant height and length of the growing cycle. The concept of landrace is complex (Zeven, 1998), and here we use this term for a locally grown maize population that a farmer cultivates and manages as a seed lot. A seed lot is defined as "…all kernels of a specific type of maize selected by a farmer and sown during a cropping season to reproduce that particular maize type" (Louette et al, 1997:24). However, for maize a very specific taxonomy has been proposed and used extensively, based on the concept of races of maize. Anderson and Cutler (1942) were the first to propose classifying maize by "races". Wellhausen et al. (1952) refined this concept and used it to describe the diversity of maize types cultivated in Mexico. Currently, 59 races have been described in Mexico (Sánchez et al. 2000b). The book «*Races of maize in Mexico, their origin, characteristics and distribution*» by Wellhausen et al. (1952) set up the fundamental concepts for

the analysis of diversity of maize in Mexico, on the American continent, and on other continents. The concept of race was not only used by these authors for descriptive purposes but also to propose hypotheses on the origin of these races and their relationships. These hypotheses relied on a series of assumptions, both explicit and implicit.

While these authors defined a race according to the definition of Anderson and Cutler (1942), i.e., 'a group of related individuals with enough characteristics in common to permit their recognition as a group,' they recognized that 'perhaps the majority of varieties collected in Mexico are mixtures of 2 or more races' (p. 44), implying that their classification excluded the majority of the collected samples. Ortega (2003 p. 132) also recognized that most populations represent combinations of races. Wellhausen et al (1952) proposed a phylogeny (derivation) of races based on the following hypothesis: 'A race is suspected to be of hybrid origin when it is clearly intermediate in certain of its ear and plant characteristics between two races, which occupy adjacent areas or overlap in their distribution or when there is a good reason to believe that they have done in the past' (p. 43). This hypothesis is valid only if there is no or very limited variation within groups (races), because in this situation an intermediate form can be only obtained from hybridization between 2 other different forms (races, populations, etc.).

Since the publication of this book, information has accumulated and new analytical tools have been made available. These results challenge the assumptions the authors just mentioned relied upon. A genetic study based on neutral markers (Matsuoka et al., 2002) showed that races are organized as a continuum and that their differentiation is mainly due to isolation by geographic distance. In a genetic study of landraces in Oaxaca (Mexico), Pressoir and Berthaud (2004a) showed that neutral genetic diversity is high but it is not structured among populations in this local area. Instead, each maize population holds almost the same diversity, suggesting that gene flow among maize populations is important in this region of Mexico. They also showed that management of seed by farmers maintains strong phenotypic differentiation among maize populations. In other words, local landraces are recognizably different even though genetic studies show that gene flow among them is common. These results lead us to propose the hypothesis that farmers, through their constant management of seeds and landraces, play a large role in the differentiation of populations and races of maize through divergent selection – a hypothesis not considered by Wellhausen et al. (1952).

Goodman and Brown (1988) also described races and their distribution on a regional scale. They developed the concept of race groups, i.e., clustering races according to similarities for some traits. They were able to cluster Mexican maize races into three groups. One group of races has long and narrow ears, and is found in the Northwest of Mexico and in the USA. Another group clusters the conically eared races, found in Mexico at high elevation. A third group, with large to small ears is found in the lowlands, either along the Atlantic or the Pacific coasts. This classification reinforces the dichotomy between lowland and highland maize types, which extends also into Guatemala (Bretting and Goodman, 1989; Doebley, 1990)

Other indicators of genetic diversity
Genetic diversity can be detected by several other types of molecular markers. The markers related to organelles in the cytoplasm (mitochondrial or chloroplast DNA) give information on the female lineages in many plant species. Weissinger et al. (1983) used mitochondrial DNA, to show that, while most samples from races of maize in Mexico belong to the same group, which also includes samples from South America, many other groups are found in South America. This analysis showed also that the plants with cytoplasmic male sterility have distinct cytoplasms.

One male sterile cytoplasm (cms-S) was found in the race Cónico Norteño in Mexico and also in some teosinte accessions (see point 3 in this chapter). With regard to other genetic elements, the maize genome is characterized by the presence of many transposable elements (Bennetzen, 2000). These elements may promote variation in the genome of maize, in particular after various maize types or maize and teosinte have hybridized (Gutiérrez et al, 1998; Mangelsdorf, 1985).

To conclude, diversity is also a multidimensional concept that can be applied to a population of plants, a group of populations, a plant species, and to specific traits of interest. The diversity of traits observed in local maize populations is the result of farmers' interests and actions (see below). This diversity can be detected by examining the classification system that farmers use to describe local landraces (see Bellon (2001) for examples from Santa Ana Segache, Oaxaca and Vicente Guerrero, Chiapas, and Arias et al. (2002) from Yaxcabá, Yucatán). Describing diversity of maize is a complex task. Using the concept of race as described by Wellhausen et al (1952) could lead to a misinterpretation of evolution (past and current) of maize in Mexico. In some case it could induce the rejection of part of the current diversity, the part which would represent intermediate types between typical races.

2-The evolving nature of these populations over time in Mexico; genetic and agronomic factors in their maintenance: Role of hybridization and introgression in the process

Modern agricultural systems are organized around various activities carried out by many specialized players: breeders create new varieties, seed companies distribute seeds, and farmers grow crops and sell their product to agro-industry. Consumers are at the end of this chain and have access to the processed and final products. In traditional agricultural systems, farmers play many of the roles described for modern agriculture. They trade and conserve the genetic resources, they select for desirable plant traits, they plant seed from their varieties, and after harvest, they are often the main processors and consumers of their products In Mexico, these two systems co-exist but the traditional, subsistence-oriented system is much more widely distributed (see chapters 1 and 6). In the traditional systems, landraces are the result of continued evolution, as we describe below.

Factors affecting the seed dynamics of landraces:
We present here some factors that have the strongest impact on the evolution and maintenance of landraces.

Seed recycling: Saving seed from one season to the next (also known as seed recycling) is an almost universal practice among small-scale Mexican farmers, and is especially true for maize. It is their main source of seed. Farmers usually follow strict procedures to select seed to retain for the next season (Anderson 1947; Wellhausen et al. 1952). Farmers save seed not only of landraces but also of improved varieties (open pollinated or hybrids), a practice that is much more prevalent than generally believed (Ortega, 1973; Morris et al. 1999a, b). In traditional agriculture, landraces (populations) are often maintained for many growing seasons unless crop failures lead to local (on-farm) extinction. (This local extinction is mitigated by seed flows, see below.)

Seed recycling is accompanied by ongoing selection, which has important genetic implications. Selection defines which individuals pass their genes, and hence their traits and

alleles, to the next generation, thereby affecting the genetic structure of the population. Because seed is generally selected in the household and not in the field, farm families exert direct selection pressure on ear characteristics but only indirect pressure on related plant characteristics such as plant height and seed yield, which are rarely taken into account (Louette & Smale 2000; Smale et al. 1999). Some exceptions exist like in Yucatán where 20% of the seed is selected in the field (Yupit Moo 2002). At least from the farmer's perspective, seed selection may also be fundamental to maintaining the integrity of a landrace, which can be lost easily through hybridization (Bellon and Brush 1994; Louette et al. 1997). In contrast, on farms with large-scale industrial agriculture, new maize seed is bought for planting each year, because only F_1 hybrid seeds have maximum levels of hybrid vigor. On such farms, no evolution of varieties is occurring due to selection by farmers.

Seed flows: Besides maintaining seed from their own stocks, Mexican farmers commonly acquire seed from other farmers or sources within or outside the community. For example, Zapalote chico, a tropical maize race found at sea level, has been introduced from the Isthmus of Tehuantepec (sea level) into communities of the Central Valleys of Oaxaca, 200 km away and at 1,800 meters above sea level. Seed exchanges, which we refer to as seed flow, may account for almost half of all seed planted (Louette et al. 1997).

There are several reasons for seed flows. Sometimes, farmers lose seed to pests, diseases, drought, frost, and other problems and have to introduce new seed on their farms. Farmers also like to experiment, planting small quantities of foreign seed to assess its performance under their local conditions and management (Badstue et al, 2003). In some cases, they may plant "small areas" (a few thousands square meters) because of socioeconomic constraints or because they want to harvest only a small amount of a particular landrace. If those crops fail, farmers easily find themselves without seed (Aguirre Gómez 1999; Louette et al. 1997). Seed flows are also encouraged by the common belief that seed must be changed regularly to maintain productivity, enabling farmers to "sow the same maize type but from new seed" (Louette et al. 1997:31-2). As Louette et al. report, seed renewal is quite variable in time and quantity.

Seed flows are important for understanding the dynamics of diversity in a given location because they are the basis for incorporating new populations into the farm and obtaining seed of populations that have been lost but are desirable. They may be an important mechanism for the migration of genes (Louette et al. 1997).

Pollen flow: Maize pollen is transported by wind and cross pollination is almost the rule in this crop. Pollen flow permits genetic exchange between landraces planted in neighboring fields. Many of the existing studies on pollen flow measured distances of pollen flight . It has been shown that a large part of the pollen produced by a plant is dispersed within a radius of a few meters. However, a few pollen grains can be found at much higher distances. The complexity of pollen flow in landraces is compounded by plant-to-plant variability of flowering dates.

Adoption, colonization and extinction: Besides the selection process conducted by farmers to maintain or modify the type of their seeds, farmers also sometimes introduce and adopt new and different types of maize, and also withdraw some of their seed. Their choice is based on the information they may collect outside their farm, looking at their neighbors' fields or getting comments from their relatives or any other trusted person. All these events in a population's life

are elements of the selection process involved in the evolution of these populations (Ortega 1973, pp.140-142; Perales et al, 2003b).

Hybridization between maize types

Current: Observations of collections made in the Central Valleys of Oaxaca (Mexico) show that in the area of Sola de Vega, farmers introduced Tuxpeño type maize and have hybridized it with their Bolita type maize (F. Aragón Cuevas, pers. com.). Creolized (that is, "hybridized") Tuxpeño was also found elsewhere in the Central Valleys, and was selected as an elite landrace during the process of participatory plant selection conducted in Oaxaca (Bellon et al, 2003). This is due to its specific traits, close to that of a commercial hybrid maize but with a better local adaptation to climate, and to farmer practices. In another village, we (J.B) detected the introduction of samples of the Zapalote chico race type, which means that a maize type adapted to lowland is brought on a more or less regular basis to a new area of cultivation, the area of race Bolita, at 1800 meters above sea level, demonstrating that not all introductions are made from the best adapted material. Farmers are willing to experiment (Badstue et al, 2003) and to allow for adaptation through recurrent hybridization with the local genetic material. Investigating the social relationships and exchange of seeds in the same area, Badstue et al (2003) interviewed farmers. They showed that the main reasons to exchange seeds are as follows: commencing to farm is the reason given for 28% of the seed exchanges, 19% for lack of sufficient seeds, and in 32% of the cases, the highest frequency, they exchanged seeds for experimentation. After experimenting, farmers may decide to keep this seed as a new landrace on their farms, to mix it with others, or to abandon it. In any case, this willingness to experiment shows us that these traditional farmers are far away from maintaining landraces as static entities. Purity, or integrity may have a strong content in terms of perception by farmers, but the way farmers manage their populations show us that genetic purity or integrity are not strong determinants for the creation and maintenance of landraces.

  The process of creolization has been well studied elsewhere in Oaxaca and in Chiapas (Bellon et al, 2003b): "Most people consider recycled or "*acriollado*" seeds to be *criollos* in a few years. …. Key to classification as a *criollo* seed is that the seed has been "acclimatized" to local soils, i.e. seen as adapted to these soils. According to one farmer in Chiapas: "*at first it was like a hybrid and now, later, it is criollo... It likes the soil. It acclimated.*" When asked whether this process was what makes a variety 'criollo,' another farmer said "*yes, that is exactly what makes it criollo. After some seasons it adapts and will produce any place. Because they planted it once and now it knows the land and since the land is good [it produces].*" Farmers cultivate side by side various types of maize with various grades of creolization. The authors did not observe a direct replacement of landraces by the introduced modern varieties. What they observed was a genetic inter-gradation between modern varieties and local landraces, considered to have been in the area for a long time.

Future: These farmers through changes in their cultural practices could introduce more new modern maize varieties such that they make up a larger fraction of the local germplasm. A threshold could be reached such that gene swamping from the modern varieties could reduce or eliminate the genetic diversity of the local landraces. This could be possible when introduction of modern varieties becomes a permanent process and large quantities of seed of modern varieties are used every year (Linder et al., 1998, Ellstrand et al., 1999; Papa and Gepts 2003).

A major result from these studies is that the concept of local landrace is not linked in the farmers' mind to a concept of genetic integrity. A landrace exists because it has been cultivated for a time "long enough" to be locally adapted. Farmers perceive that introducing new maize types and permitting gene flow between the different maize types is a process allowing for maize evolution and adaptation. They do not consider this process as a "contamination." Although landraces may appear to represent ancient varieties that are passed on from one generation of farmers to the next, it is important to recognize that the genetic composition of landraces is in a constant state of flux.

Dynamics of genetic diversity of landraces
The characteristics of management of landraces by farmers allow for hybridization, gene flow, and multi-generational populations. They make possible a landrace evolution based on the forces that structure natural populations of wild plants, i.e. recombination, drift, migration, and selection. In long-lived populations, through recombination (see endnotes 1-3), genes belonging to a specific variety can migrate into a new genetic background—that of the local population. This local population can accept genes from various exogenous varieties.

According to population genetics theory and case studies (for example, in sunflower; see Whitton et al. 1997), when a selectively neutral gene is introduced into a population, it will remain in the population at the same frequency as when it was first introduced. Two forces can cause its frequency to vary from generation to generation: genetic drift (chance effects due to small population size and the randomness of meiotic segregation) and migration. Drift generally applies only to small populations and is independent of selection. Migration among populations ("gene flow") will tend to homogenize their allele frequencies. A study in sunflower showed that a large proportion (0.31 to 0.38) of alleles from the cultivated varieties was introduced into adjacent wild populations over several years of gene flow (Linder et al., 1998). When genes are not neutral—in other words, they have a positive or negative selective effect, selection will drive changes in the frequencies of these genes. The more favorable the gene (allele), the more rapid its frequency increases in a population. The importance of human selection—not just natural selection—in the process of crop evolution should be emphasized. Modern varieties have to comply with the several requirements to enter the commercial seed system (as defined by the Plant Variety Protection regulations) (Gepts, 2004b), i.e. they have to be distinct, uniform, stable and non-essentially derived. Distinct means a variety is different from all the other known varieties by at least one characteristic (agronomic or not). Uniform means that all plants from a variety have the same characteristics. Stable means that a variety will exhibit the same characteristics from year to year. Lastly, a variety cannot be derived from another variety and be distinct from that variety by a single gene, such as might be achieved by backcrossing or genetic engineering. These requirements are difficult or impossible to meet by the local landraces managed by farmers, because these farmers do not have much interest in uniformity and stability of their populations.

Difficulties in the detection of genetic changes in the local maize populations
To conclude our point, we emphasize the difficulties related to evaluating changes in diversity and correlating them with other factors. Individual farmers or groups of farmers have different interests. They value different traits (many not commercially valuable) and different options for the same trait. Small-scale maize farmers provide long lists of traits that they value, mainly related to agronomic, consumption, and management characteristics (see Bellon and

Risopoulos 2001; Smale et al. 1999). Evaluating this diversity as farmers do is difficult and requires the use of social and biological methodologies, as well as sampling farmers' fields and undertaking agronomic evaluations on experiment stations (see Berthaud et al, 2002; Bellon et al. 2003a; Pressoir and Berthaud 2004b).

Another difficulty in evaluating changes is the high genetic diversity detected within many of these populations. Pressoir and Berthaud (2004a) found that the neutral genetic diversity in the populations of a few villages from the Central Valleys of Oaxaca is almost equivalent to the total maize diversity found on the American continent. This genetic diversity is mostly found within populations, rather than distributed evenly among them. It results from the interplay of forces described above.

An additional difficulty in the analysis of this diversity and its changes arises from the fact that different markers (morphological and molecular) relate different information about the partitioning of diversity because they reflect different processes involved in the evolution of populations. Pressoir and Berthaud (2004b), analyzing the diversity of maize in the Central Valleys of Oaxaca, showed that, whereas the same neutral diversity (detected through molecular markers) is found in each population (that is, among-population variation is very low), morphological diversity is found within and among populations. As mentioned earlier, neutral diversity reflects the demography and history of maize populations. The morphological diversity observed in local maize populations reflects the history of farmer selection and natural selection. And lastly, changes in diversity do not have a linear impact, i.e. thresholds in frequency of alleles or traits could exist that trigger very dramatic qualitative changes. These non-linear dynamics are a very interesting field of research.

3- Maize and teosinte hybridization and introgression, past, present, and future

Introgression is a process that follows hybridization and leads to the integration of a limited number of genes or genome regions from a donor taxon (e.g., maize) into a recipient taxon (e.g., teosinte). Evolution of teosinte through gene introgression from transgenic or non-transgenic maize could result from a chain of events or occurrences (Gepts and Papa, 2003):

A)- Existence of areas where maize and teosinte are sympatric;

B)- Pollination and fertilization of teosinte by maize. This implies some overlapping of flowering periods and compatibility between maize and teosinte genotypes;

C)- Once F_1 hybrids occur, successive generations of backcrosses take place to recover teosinte plants with introgressed genes from maize;

D)- If some teosinte plants are successfully introgressed with genes from maize (transgenic or not), these plants will become established if their selective value gives them an advantage over the non-introgressed forms;

E) Modifications of wild relative through gene flow and introgression will also depend on the various characteristics of the gene flow which can be permanent, rare, and frequent.

Examples of maize-annual teosinte co-occurrence

Balsas area

Wilkes, (1967, p 72) described the different situations occurring for the various races of teosinte. In the Balsas region (State of Guerrero) where *Zea mays* subsp. *parviglumis* is found, teosinte is a wild plant that is very well established in many places, along roads, and outside

maize fields. Wilkes recognized both spatial and temporal isolation of teosinte from maize. In this region, teosinte flowers 2 to 3 weeks later than maize. However, when teosinte is found along maize fields, F_1 hybrids are detected. Sánchez and Ordaz (1987) reported that in their collections of Balsas teosinte, 10 out of 55 populations exhibited teosinte x maize hybrids, at least morphologically. Clearly, in this area, there are situations where teosinte and maize are not as isolated as was previously reported.

Valley of Mexico

In the valley of Mexico, teosinte belongs to race Chalco, a representative of *Zea mays* ssp. *mexicana*. In this area, teosinte has all the traits of an agricultural weed. It is found almost only in and along cultivated maize fields. When populations of teosinte are observed outside the fields, even right at the edge of the fields, they generally do not survive to the next planting season. In some fields, teosinte accounts for up to 10% of the plants (Wilkes, 1967). Thus, there is no spatial isolation. Temporal isolation is "only partially operative" (Wilkes, 1967, p. 73). Flowering of teosinte is about two weeks later than maize. However, Kato (1984) mentioned that flowering periods "occur in perfect coincidence." Wilkes (1967) very well described the distribution of F1 hybrids in the maize fields and noted a proportion of F_1 hybrids of 2-5% of the teosinte plants.

Central Plateau

According to Wilkes (1967), teosinte found in this area (northern part of the state of Michoacán and southern part of the state of Guanajuato) belongs to the race Central Plateau, which is also part of *Zea mays* ssp *mexicana*, the same subspecies as in the Chalco area. This subspecies also includes the populations discovered around the town of Durango (Doebley, 1983). In this region, populations of teosinte are found wild or as weeds. Teosinte plants grow in maize fields and also in places where there is no maize (Wilkes, 1967 and J.B., pers. observ.). There is no spatial isolation, but there is temporal isolation as teosinte flowers two weeks after maize on average.

It is worth mentioning that this co-occurrence is not only due to the common adaptive traits of the various teosintes and maize but is also the result of farmer's management of their seeds and fields. Teosinte is already considered a weed in some parts of Mexico and farmers would prefer to eradicate it. It is maintained in some fields because some farmers do not invest very much effort in weeding. But one can imagine that introduction of herbicide sprays at some step in the cropping system could lead to the eradication of this weed (and the local extinction of teosinte). In the Chalco area, teosinte is also maintained because farmers use teosinte as fodder, the teosinte seeds survive in the intestinal tract of cattle, and manure is used for fertilization of new maize fields. Through this management it is easy to have new teosinte populations become established and invade new fields. However, changes like use of chemical fertilizers or herbicide will surely restrict the occurrence of teosinte.

Factors affecting hybridization between maize and teosinte

Pollination of teosinte by maize: As already mentioned, the Mexican annual teosintes are generally temporally isolated from maize. However, the duration of flowering of teosinte is quite long (J.B., pers. observ.) and it is easy to find some plants with a flowering period that overlaps with that of maize. Some teosinte plants even flower before maize in the Chalco area. In fact, germination of teosinte is triggered by rains, the distribution of which varies from year to year.

Farmers do not all plant at the same date and landraces of maize can flower over several weeks. To estimate gene flow, one will have to consider variation from field to field and from year to year.

Fertilization: It has been long known that Chalco teosinte has an incompatibility system that limits gene flow from maize. This system works as a barrier for crosses from maize as the male parent to teosinte as the female parent, but is inefficient in the reciprocal cross. The barrier is strong, but not absolute, as it is still possible to produce F_1 hybrids with teosinte as the female parent (Allen et al 1989). Kermicle and Allen (1990) found that, in this area, most of the teosinte plants carry the *Ga1* allele. This incompatibility system works to isolate the teosinte, preventing fertilization from maize pollen, which in many cases does not carry a *Ga1* allele. In order to estimate the efficiency of this system, we should know the frequency of *Ga1* in teosinte populations and in sympatric maize, data that is currently unavailable. It has to be noted that *Ga1/ga1* heterozygotes will accept *ga1* pollen but with a low success because ga1 pollen grains are at a disadvantage when they have to compete with *Ga1* pollen. It is also possible for the F_1 hybrids (*Ga1/ga1*) to pollinate teosinte plants. These F_1 hybrids could be one of the relays needed to move genes from maize to teosinte. It is also possible that this system is more genetically complex, consisting of more that one gene. In the Central Plateau area, Kermicle and Allen (1990) and Evans and Kermicle (2001) demonstrated that at least two genes are involved in the incompatibility system, one allelic to *Ga1* and another, a new locus called *Tcb1* (Evans and Kermicle, 2001) located on the same chromosome 4. Frequencies of the incompatibility alleles in Central Plateau teosinte populations are not known and neither is their possible occurrence in maize from the same area. According to Kermicle and Allen (1990), there is no incompatibility gene in teosinte from the Balsas area. However, they mentioned that the *Ga1* allele could exist at a low frequency. In their study of the F_2 generation of maize x Balsas teosinte crosses, Doebley and Stec (1993) found that their teosinte progenitor had the *Ga1* allele.

Does hybridization and introgression occur between maize and teosinte?
This question is quite controversial and only very limited set of data is currently available (Serratos et al, 1996). The presence in the fields of apparent F_1 hybrids between maize and teosinte (based on morphology) is well documented (Wilkes, 1967; Sánchez and Ordaz, 1987) while information is lacking about the behavior of the advanced hybrid generations. Wilkes (1967, p 80-81) mentioned that in some small teosinte populations plants exhibited non-brittle rachis and paired spikelets, which are considered maize-specific traits. This observation suggests that introgression from maize occurred in these small populations and teosinte populations can survive with some domestication traits. Doebley (1990) presented a convincing example from *Z. diploperennis*. He found a plant with alleles from maize at two linked loci. Frequency of these alleles was 0.01. In contrast, Kato (1984) was not able to find knob distribution in maize and teosinte (*Zea mays* subsp. *mexicana*) that would have suggested introgression in the Chalco area. For example, abnormal chromosome 10 type II is only found in teosinte and not in sympatric maize.

It is important to note here that we are also facing a methodological problem. Maize and teosinte are very close geographically and genetically. We can expect they still share a lot of alleles just by common ancestry. On the other hand, if hybridization and introgression are occurring at a very high rate, we can expect both maize and teosinte to have comparable allele frequencies. In order to detect introgression very specific analyses have to be conducted, for

example, to estimate linkage disequilibrium between different genes in different situations of possible hybridization.

A case of possible introgression that we consider worth studying in more detail relates to the male sterile cytoplasm *cms-S*. Some populations of teosintes share the same male sterile cytoplasm *cms-S* as some maize populations (Doebley and Sisco, 1989; Weissinger et al, 1983). This provides an example of probable inter-subspecific gene flow that occurred through several generations of backcrosses carrying the same female cytoplasm through several generations of unadapted materials. An alternative explanation would be a transfer of cytoplasm through the male gamete. Transfer of chloroplasts has been demonstrated for another cereal, *Setaria italica* (Wang et al., 2004). Whatever the correct explanation, this is an example of a gene transfer that some considered nearly impossible (Martínez-Soriano and Leal-Klevezas, 2000). In any case, it would be very useful to document the different steps of the introgression and to explore if it can be linked to particular practices of the farmers or to demonstrate that cytoplasmic organelles can be transmitted through pollen.

Results from Blancas et al. (2002) have shown that introgression can occur between cultivated maize and teosinte: 'Our data provide evidence for introgression of maize and teosinte in that sympatric teosinte populations are more genetically similar to maize than to allopatric teosinte. This observation seems to contradict evidence (Doebley et al. 1984) that sympatric teosinte maintains allozymic distinctness from maize (Doebley, 1990). It seems instead that there is genetic exchange between maize and teosinte growing in close proximity or sympatrically.'

When we search for the effects of hybridization and introgression we have to consider that effects of hybridization are not limited to the introgression of crop alleles in a wild background or the opposite. They may occur from the interaction of different genomes in the hybrids and their advanced generations, which may represent new genetic combinations. It has been shown in some cases that this stress contributes to the activation of "dormant" transposable elements, then producing new transposition and new mutations (Grandbastien, 1998). A similar hypothesis had already been proposed by Mangelsdorf (1985) in the case of maize x teosinte hybrids. This activation relates to the state of silencing or unsilencing of genes in the genome and can be more complicated when hybridization occurs between transgenic maize and teosinte, as transgenes are affected by silencing (for a review on silencing, see Fagard and Vaucheret 2000).

4- What is the effect of introducing transgenes in the maize genetic diversity dynamics?

Based on the information presented in the previous section, we assume that gene flow is taking place and that transgenes will be introduced into the gene pools represented by local maize landraces and teosinte. We also assume that transgenes are generally expressed as expected in their new genomic background with the caveat expressed in the next section on Stability of transgenes. What are the potential effects of such introduction? Three main topics need to be discussed: 1) to what extent do the transgenes have an effect *per se* because they affect the fitness of the recipient individuals and, therefore, can affect selection operating on these individuals; 2) stability of transgenes in different genetic backgrounds and environments; and 3) to what extent do transgene have an indirect effect on genome regions adjacent to transgenes or even the genomes as a whole as a result of selection and migration?

Potential selective effect of transgenes in different genetic backgrounds and environments
An insect resistance gene such as the Bt gene may, for example, increase the fitness of either landraces or teosinte if their growth and yield are currently constrained by insects controlled by the Bt gene. A first step in evaluating this possibility is to determine which insect predators currently affect maize and teosinte in Mexico and if resistance conferred by Bt would confer some advantage to either taxon. A non-exhaustive review of the available literature suggests that one of the main maize pests is the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae). Another insect pest is the stalk borer, *Diatraea lineolata* (Walker) (Lepidoptera: Pyralidae) (Smith et al. 1987; Mugo et al. 2001; Penagos et al. 2003). Because these are Lepidopteran insects, they are presumably susceptible to the lepidopteran-specific Bt toxins that are most prevalent in currently available commercial varieties of Bt maize. Selection exercised by these insects may lead to an increase in frequency of the Bt transgene in landrace and teosinte populations. However, this is by no means certain and should be verified empirically.

Studies on transgenic sunflower in the United States are particularly illustrative in demonstrating why experiments are necessary. Snow et al. (2003) showed that the transgenic Bt gene increased fecundity in wild sunflowers, whereas Burke and Rieseberg (2003) showed that the oxalate oxidase transgene (which confers a resistance to white mold caused by *Sclerotinia sclerotiorum*) had no effect on fecundity of wild sunflower. Whether wild sunflower benefited from a transgene depended on whether it already had some level of resistance to the herbivore or disease or whether these biotic factors were inherently limiting fitness of wild sunflower or both. The distribution of seed weevil resistance factors in common bean shows that selection pressures exercised by insects (and diseases by extension) can be quite different in wild and domesticated populations (Gepts and Papa, 2003). These observations demonstrate that beyond the issue of gene flow, the effect of transgenes on fitness in the recipient populations should be measured to fully assess the impact of gene flow of transgenes. This analysis does not lend itself very well to predictions and extrapolations and should, therefore, be performed on a case-by-case basis.

If herbicide tolerance genes are introduced into other genotypes, these in turn may become herbicide tolerant. This could be of benefit for local farmers, if they can and are willing to use herbicides and if the patent owner tolerates this inadvertent escape without taking legal action against the farmers in question. If herbicide tolerance is introduced through gene flow into teosinte, then a tool for control of teosinte in maize fields is lost. Further information on herbicide use in Mexican agriculture is provided in Chapter 5.

Whether or not a transgene will spread into and persist in landraces or wild populations depends on a number of factors, including principally the level of gene flow in any given growing season and in successive seasons, and the selective effect of the transgene (Lenormand, 2002; Haygood et al., 2003). Information on the year-to-year and location-to-location variation of gene flow is rare. In addition, the selective value of a transgene in wild populations may or may not be similar to that in domesticated populations. If a gene is introduced at a very low frequency (on the order of the mutation rate, that is, <<1%) and has no or only a slight selective effect, whether it is a transgene or not, its most likely fate is extinction in a few generations. Calculations by Crow and Kimura (1970) show that the probability of survival of neutral mutations is less than 20% after only 10 generations. Whether a gene with a selective advantage will survive in a population depends on its initial gene frequency. If it is very low, even advantageous may be lost to genetic drift. Further considerations include the degree of dominance, the presence of epistatic interactions, and the existence of genotype x environment

interactions. Depending on the magnitude of these different evolutionary factors, the situation faced by transgenes may amount to a migration-drift or migration-selection balance (Gepts and Papa, 2003). Table 2 shows the outcome in terms of gene frequencies of different population genetic contexts. Over the evolutionarily short time scale considered here, mutation is not a major factor but migration, selection, and drift are.

Table 2. Outcome of different population genetic contexts on gene frequencies

Population genetic context	Effect on gene frequencies
Infinitely (very) large population with random mating; no mutation, migration, selection, drift	Hardy-Weinberg equilibrium; in one generation, <u>constant genotypic frequencies</u>
Small populations	<u>Genetic drift</u>: random variation in gene frequencies may lead to fixation (k=μ) or loss of genes (even in large populations: probability of loss = 0.37)
Small populations + migration:	
• m > 1	• migration overcomes population drift: no population differentiation
• m < 1	• population differentiation maintained
Selection (positive):	
• very low gene frequency (similar to mutation rate)	• <u>genetic drift</u>: usually loss: fixation probability of similar magnitude to selection coefficient (≈ *2hs*)
• medium or high gene frequency	• <u>selection</u>: increase in frequency
Selection + migration	
• migration > selection	• <u>fixation</u> of migrant alleles even if s < 0 (→ demographic swamping)
• migration < selection	• <u>population differentiation</u> maintained

The two situations that can lead to a displacement of genetic diversity by an immigrant allele are high levels of migration or a strong selection effect (and the combination thereof). Only in this situation will allele frequencies of genes imported from industrial or bred cultivars come to predominate in the traditional or local varieties and teosinte populations. If migration is sufficiently strong, even genes that are not adaptive can become fixed (Haygood et al., 2003).

Stability of transgenes
During the review process of this chapter the topic of stability of transgenes has been much debated. There are two types of stability to be considered. The first is structural stability, in which the integrity of the original construct and its insertion in a specific location of the genome ("event") is considered. The second is functional stability, in which the level of expression at various levels (messenger RNA, product) is considered. Furthermore, stability of transgenes has

to be considered at all stages of the genetic transformation process and after the diffusion of the commercial transgenic varieties. Literature which describes the stability problems encountered through the transformation process exists (Padgette et al, 1995; Zhong, 2001; Vain et al, 2002). Stability is also a requirement for the approval of commercial transgenic varieties. However the latter type of data is not easily accessed by the public. In the 2003 annual report of the ARS project on quantitative genetics and grain quality of corn (Scott Malvin coordinator, http://www.ars.usda.gov/research/projects/projects.htm?ACCN_NO=403995) it is noted that: « Until recently, there have been no transgenic corn plants available to the public sector, so studies of transgenes at the level of basic science have been severely limited. These plants will allow us to investigate many problems associated with transgenics that have been overlooked by industrial researchers. These problems include the impact of transgenes on the genome, inheritance and stability of transgenes, and transgene silencing.» Requests to three different seed companies for isogenic pairs of transformed v. untransformed maize lines have been denied by these companies (P. Gepts, pers. observ.).

Padgette et al. (1995) described the great length necessary to develop a transgenic line of soybean. Zhong (2001) presented a general overview of the difficulties of integrating the transgenic process in the mainstream plant breeding program due to the need of selecting stable insertions and Vain et al (2002) continued this discussion. Gahawka et al. (2000) documented a high level of variation in the expression of certain transgenes among sister lines and claimed that this variation was heritable based on a visual correlation in expression between parents and progeny. The presentation of the data, however, lacked quantification of the expression over all lines, as is achieved in parent-offspring regression calculations, a standard technique to measure heritability in plant breeding.

From the papers cited, it is clear that transgenic events have to go through a process of selection to verify stability in a given genetic background and over a range of environments. We do not dispute that the commercialized lines that result from this process probably show stability. The issue which is raised is the following: if one changes the genetic background or the environments, what happens then? Is stability maintained? Extensive literature searches conducted by the authors have failed to reveal peer-reviewed, publicly available data that satisfactorily address these questions. Experience from plant breeding shows that drastic changes in genetic background or environment could possibly lead to instability of expression. Many such cases may not be readily published because they constitute negative results that detract from the goals of plant breeding. The issue of negative results and its under-representation in publication is a broader issue beyond this report.

Potential effects of transgenes on genome diversity
With regard to the second topic, a transgene subject to selection may affect the rest of the genome as well. If the selection is positive (*i.e.*, individuals containing the transgene will be favored), the genome will be subject to a *selective sweep* or *hitchhiking* at the locus and surrounding linked genome regions. If the selection is negative, the genome will be subject to *background selection* (Charlesworth et al., 1993). In both cases, selection will lead to reduced diversity at the locus and adjacent genome regions. The size of the affected region subject to reduction in genetic diversity ("genomic window") is proportionate to s/r, where s is the selective advantage of the gene under selection and r is the level or recombination.

For outcrossing individuals, characterized by high levels of heterozygosity and effective recombination, the region of the genome that remains linked around the transgene can be very small (of the order of 500-100,000 base pairs (bp) out of a total genome size of 2.7 x 10^9 bp in maize: Bennett & Leitch 1995; Wang et al., 1999, 2001; Remington et al., 2001). A recent article by Clark et al. (2004) provides a better picture of the size of a genome region that is affected by selection 5' upstream of the *tb1* gene, responsible for the lack of branching in maize. It is clear that the *tb1* gene has been under strong selection ($s \approx$ 0.04-0.08: Wang et al., 1999) during maize domestication, as domesticated maize has a single stem, in contrast with teosinte, which is generally branched. According to Clark et al. (2004), the size of this region is about 50,000 bp up to 90,000 bp. Given that the size of the maize genome is 2.7 billion bp, the region affected by selection affecting the *tb1* gene is approximately one hundredth of one percent. Thus, the region showing reduced diversity as a result of selection on a particular gene would be small compared to the entire maize genome. The only circumstance in which this conclusion may not hold is when high levels of gene flow may overwhelm the effects of selection (see previous section).

Another consideration to take into account is the fate of the transgene within the genome. It is generally assumed that after their insertion into a genome are stable at that position. The second assertion (in addition to the presence of transgenes in Mexican maize landraces) of Quist and Chapela (2001) was that transgenes seemed to wander in the genome. Although their data did not support this assertion, it remains an open question given the lack of research data on this topic. The stability of expression associated with the major commercial transformation events has led people to assume that the transgene is inserted in a stable fashion in the genome. This is most likely the case even though no publicly available molecular data exist in this respect. What remains to be determined is whether the stability of insertion and expression will remain once the transgene construct is introduced into novel genetic backgrounds such as those provided by Mexican maize landraces and teosinte. The commercially usable transformation events are only a small fraction of those that have been assayed and retained after an elaborate selection procedure (e.g., Padgette et al., 1995; Zhong, 2001). What are the insertion characteristics of the insertion events that were not selected? Did the (relative) lack of expression, expression in "wrong" tissue, or instability of expression relate to the insertion location or mode?

The insertion of transgenic DNA may bring about small-scale rearrangements of the transgene and native DNA sequences at the insertion site (e.g., Pawlowski and Somers 1998; Svitashev et al. 2000, 2002; Windels et al. 2001). At this stage, it is not clear whether other rearrangements involving other regions of the genome, including transposition or translocation of chromosome segments containing the transgene should also be considered. After all, maize is the organism in which transposable elements were first described (McClintock, 1984). Furthermore, it is known that expression of genes (timing, tissue-specificity) depends on their location in the genome ("position effect") (Fagard and Vaucheret, 2000) and particularly the conformation of chromatin, which is largely determined by the chemical status of DNA-binding histone proteins (Jenuwein and Allis, 2001; van Driel et al., 2003). There are interactions with other genes in the genome ("background effect"), which affect the overall level of expression of the trait. When more than one sequence is introduced or if a transgene is similar to a native sequence in the genome, then gene silencing can take place (Comai, 2000; Iyer et al., 2000).

Recent studies in common bean have shown highly asymmetrical gene flow between domesticated and wild types, with a predominance of gene flow from the former to the latter (Papa and Gepts, 2003). This asymmetry may be attributed to a larger domesticated pollen mass

in comparison to that of wild types, to the recessiveness of domesticated traits (although there are exceptions), and/or to stronger selection by farmers against hybrids compared with natural selection against domesticated traits in wild environments. Many domestication traits are recessive and represent a loss of function. In addition, they represent adaptations to cultivated environments (Gepts 2004a) such that fully domesticated plants cannot survive without human intervention in the wild. Introduction of domestication genes from non-transgenic crops is, therefore, unlikely to cause severe ecological problems unless recombination of domesticated and wild traits takes place leading to weedy hybrids. In contrast, addition of transgenic traits, such as insect resistance, may actually cause in increase in ecological amplitude by releasing wild relatives from certain constraints such as insect pests.

The asymmetry in gene flow concerns about possible genetic assimilation of wild populations by their domesticated descendants. Although this is not an effect of transgenes *per se*, it is a concern related to the type of industrial agriculture in which transgenes are deployed. This type of agriculture is typically dominated by a few seed companies and, therefore, a limited number of cultivars, which represent a small fraction of the entire diversity of the total gene pool. In contrast, small-scale farmers generally maintain a range of genetic diversity on their farm because they are by necessity more self-reliant. It is this type of agriculture that may suffer from genetic assimilation and displacement of genetic diversity if they are neighbors to industrial maize fields. The same concern holds for diversity of the wild gene pool (teosinte) (Gepts and Papa, 2003).

In summary, whether or not a transgene from a transgenic source population will become established in sink populations depends on many parameters, including the magnitude of the selective advantage and the migration rate, genetic drift, epistatic effects, and genotype x environment interactions. These parameters are not inherently different from those governing the fate of non-transgenes subject to gene flow, with the exception of the novelty of transgenes, which makes any prediction more difficult given the absence of biological and ecological information on the effect of transgenes in their new genetic and environmental backgrounds.

5- Briefly review evidence of transgenes

The actual situation with regard to transgenes in maize landraces and teosinte in Mexico is clouded by a fog of claims and counterclaims based on a lack of peer-reviewed data, with the exception of the initial paper by Quist and Chapela (2001) and the ensuing replies. Although the Mexican government has set up additional sampling and analyses to evaluate the magnitude of the geographic distribution of potential transgenic sequences, there have been government communications (Enciso L., 2002) but the results have not been published in a peer-reviewed journal. In fairness to the Mexican government and the researchers who conducted the experiments, a follow-up manuscript containing results of these analyses was submitted to the journal *Nature* but apparently rejected (Enciso L. and Morales, 2002).

Table 3. Summary of empirical studies conducted to assess presence of transgenes in Mexican maize landraces.

Source	Date	Organization	Laboratory	Plant materials[a]	Analyses[b]	Result	Peer-revie-wed
Quist and Chapela (2001, 2002)	29 Nov. 2001, 11 Apri. 2002	UC Berkeley	Chapela, UC Berkeley	4 landraces (bulks) of OAX: 600-2,400 plants; 1 sample from Diconsa store	DNA (PCR): 35S	Positive	Yes
Alvarez-Morales (2002)	October 2002	CINVESTAV, Irapuato	Several laboratories in Mexico	Landraces of Oaxaca and Puebla	1) DNA (PCR): 35S, NOS terminator or cry genes; 2) protein (ELISA): PAT, ESPS, Cry1A and Cry9C	Positive for Cry1A, negative for Cry9C	No
CIMMYT (2002)	17 Oct. 2002	CIMMYT	Biotechnology, CIMMYT	105 landraces from the gene bank: 1,200 plants as bulks	1) DNA (PCR): 35S, PAT 2) Phenotypic: herbicide resistance (Roundup, Basta)	Negative Negative	No
ETC (2003)	9 Oct. 2003	ETC and other NGOs	Fumigaciones y Mantenimiento de Plantas SC	1) Jan. 2003: Landraces of CHI, MEX, MOR, PUE, SLP, VER; 520 plants in 105 bulks 2) July 2003: CHI, DUR, OAX, PUE, TLA, VER	Protein: Bt (4 events), ESPS	Positive	No

[a] CHI: Chihuahua; DUR: Durango; MEX: Mexico state; MOR: Morelos; PUE: Puebla; SLP: San Luís Potosí; TLA: Tlaxcala; VER: Veracruz
[b] 35S: 35S promoter of the Cauliflower Mosaic Virus; Cry: Bt protein; ESPS: 5-enolpyruvylshikimate-3-phosphate synthase, enzyme conferring resistance to the herbicide glyphosate (Roundup); PAT: phosphinothricin acetyltransferase, enzyme conferring resistance to the herbicide glufosinate (Liberty or Basta or Bialaphos);

We are now left with information from four sources: 1) Original article of Quist and Chapela (2001) and subsequent reply (Quist and Chapela 2002); 2) A presentation by Alvarez-Morales (2002) about a study conducted by the Mexican government on the presence of transgenes in landraces of Oaxaca and Puebla; 3) Statement of CIMMYT (2002) regarding the absence of transgenes in its collection; and 4) A study by several NGOs, among which ETC (2003), on the presence of transgenic proteins in several states of the Mexican republic. Table 3 summarizes some of the findings.

The consensus at this stage is that transgenic sequences are present in Mexican maize landraces in the field although not in the CIMMYT germplasm bank. This consensus view confirms the initial claim of Quist and Chapela (2001, 2002) in spite of strenuous claims to the contrary early on by genetic engineering proponents (e.g., Christou, 2002). However, this leaves many open questions, including the geographic magnitude (how widespread?), the local intensity (what is the local frequency of transgenes?), the identity of the transgenes (only those from commercialized varieties such as Bt and herbicide resistance or from as yet uncommercialized genotypes such as maize transformed for pharmaceutical production), the possible presence in teosinte, the source(s) of the transgenes (pollen gene flow from Mexican field tests conducted prior to the current moratorium, imported grain sold in local government stores, emigrants to the U.S., or seed companies), the fate of transgenes in landraces and teosinte, and the role of farmers and others in gene flow by pollen and seed, involving transgenic sequences.

Table 3 refers to transgenic traits in maize, currently achieving a substantial market share. Other traits are grown commercially on a smaller scale (up to now) or are under development. Among developments of potential concern are the utilization of corn as a "pharm" crop, i.e., to produce pharmaceutical compounds. Concerns about gene flow would be magnified many times over what they are currently, certainly in an environment like Mexico, where gene flow is a widespread and inherent part of natural and agricultural maize ecosystems.

6- Preservation of the genetic diversity of maize landraces and teosinte taxa: what do we do about it?

The genetic diversity which exists in the maize landraces arises from specific processes that we described earlier (see chapter 3, sections 3 and 4): seed recycling, seed flows, mixing seeds of different origins, and farmers' selection based on cultural and agronomic criteria. These processes are dynamic; preservation of diversity should be based on the preservation of these processes as well as the conservation of landraces per se.

Biological aspects

This conservation is currently conducted in gene banks, where seeds are preserved and a limited amount of information about these seeds is documented. A way to improve this conservation would be to strengthen the conservation of information related to the material being kept, as well as collecting information on other materials not necessarily kept in the bank but that could be collected in the farmers' fields if needed. Generating data and organizing an easy access to these data for a large public would help in creating interest for these landraces, triggering a broader use of them. A comprehensive collection of these landraces, tested for absence of transgenes (CIMMYT 2002), would also constitute a reservoir from which foundation seeds could be extracted for further planting, in case farmers request them. These foundation seeds could also be used if farmers decided to revert to GMO free landraces after adventitious transgenes have been detected.

If required, what could be the different ways to keep landraces GMO free or to return to a GMO-free status? One would be to avoid the introduction of transgenic varieties where landraces are cultivated and where farmers do want to avoid the presence of transgenes. Others, in case the presence of adventitious transgenes is detected, would be by "cleaning" the landraces of these transgenes. We can imagine doing that by gene swamping, i.e. reducing the frequency of transgenes through the repeated introduction of GMO-free landraces. These landraces could be extracted from the gene bank and returned to their original fields. This could also be done in one

step, by eliminating the transgenic plants from the reproduction of the landrace. Theoretical considerations (Crossa et al, 1989) have shown that it is possible to maintain most of the allelic diversity of a gene bank accession (or sample of a landrace) when 200 plants contribute in a controlled manner to the next generation. If transgenes are detected in a landrace, it is most probable that not all the plants will be transgenic, and through a screening process it should be possible to find the 200 plants needed for seed production.

Landraces, however, are not static entities. They are constantly evolving due, in part, to the selection pressure exerted by farmers, to adapt them to their needs from agronomic, commercial, and culinary and other end-use standpoints (Perales et al., 2003a, b). For landraces to survive, they must maintain their usefulness to farmers. We contend that establishing more effective selection schemes on the part of farmers may constitute an effective incentive for farmers to maintain their landraces. There are two, non-mutually exclusive ways in which this can be achieved. In the first way, plant breeders are somehow involved. Yet, so far there has been limited penetration of modern plant breeding cultivars in most of Mexico. This has been attributed to the good agronomic and end-use performance of landraces in comparison with breeder's cultivars. Conversely, it is difficult for breeding programs to develop materials for the multitude of niches of Mexico, each with its own combination of biotic and abiotic factor. A closer collaboration between breeders and farmers and rural communities may help bridge the gap between experiment station and farmer's fields. This collaboration – broadly called participatory plant breeding (Cleveland and Soleri 2002) – can take on several forms depending on the closeness of the interaction between the two parties (see Ortega, 2002 for a review of the past and current projects in Mexico).

Secondly, farmers can be trained to conduct some degree of plant breeding, if they do not already do so. This type of outreach would require an active extension service, a network of NGOs or local cooperatives, or a network of technical schools. In a general sense, such policies would fit in a broader policy framework stimulating rural life. The goals of such a policy would be varied but would include installation or improvement of basic infrastructure such as roads, electricity, communications, and schools and agricultural research geared to agricultural communities and smallholder farms. As part of such policies, Bellon (2004) suggest that policies be instituted that, on the one hand, increase demand for genetic diversity by increasing its value to farmers or decrease the cost to farmers of maintaining it. On the other hand, these policies should decrease the cost of accessing diversity.

Cultural aspects

Preservation of genetic diversity can rely on biological strategies but should also rely on cultural aspects. In Mexico, we recognize that maize has a special cultural value, especially because a large part of the production is for human consumption. We think that preservation of genetic diversity can be achieved through a more active use of the diverse landraces and their diverse products, especially in urban areas, where many of the maize products are currently very standardized and uniform. Education and information of urban consumers about diversity of maize and its products would help promote the use of products from these landraces, permitting the conservation of the diversity in the fields.

CONCLUSIONS

7- Questions about the future of this evolving system in Mexico and about the introduction of transgenes in this system. It is unlikely that a single or even several transgenes by themselves would affect the fate of Mexican agriculture. However, in many ways, transgenes can act as telltale sign of processes affecting agriculture of that country. For example, if it were not for transgenes, studies on gene flow, as affected by natural and human factors, would still be merely of interest primarily to scientists involved in population genetics and conservation biology. Instead, gene flow is considered to be a societally important topic because it is recognized as affecting the survival of endangered species and populations, may be affecting our supply of food and feed through the production of pharmaceuticals and industrial compounds in crops, and may play a role in the invasiveness of certain plants and animals.

The discussion so far highlights our lack of knowledge in many areas of biological and social sciences. From a biological sciences viewpoint, there is a need to better understand the process of pollen-mediated gene flow in maize (frequency, genetic barriers, distances), the level and stability of expression of transgenes in different genetic backgrounds and environments, the role and stability of the genomic context in which successful (and unsuccessful) insertion events operate, and the selective role transgenes may play. It is important to keep in mind here that many of these questions have not been studied for regulatory purposes in the U.S. because landraces are very rare and wild populations are absent in that country, in contrast with the situation in Mexico, which is the center of domestication of maize.

From a social science viewpoint, it is necessary to better understand the human factors that influence seed-mediated gene flow, the origin of the current presence of transgenes in maize landraces, and the importance of transgenic traits in traditional agriculture. Furthermore, the intellectual property aspects of gene flow in a Mexican context need to be studied in more detail. What will be the consequences for traditional Mexican farmers if patented transgenic constructs make their way into farmer's fields (with or without knowledge of the farmers)? Again, the situation in Mexico is quite different from that of the U.S. in that in Mexican agriculture, gene flow is a fact of life and even a necessity for traditional agriculture. Such aspects need to be analyzed cautiously as intellectual property legislation copied from developed countries (Gepts, 2004b) may not be the most appropriate response for Mexican traditional agriculture. Of concern is the possibility that patented gene constructs are introduced by gene flow into landraces, which may force farmers to relinquish the ownership of these seed stocks.

8- What should be done? Diversity of maize and its evolution in Mexico follow complex patterns because many variables are involved and farmer management of seed plays a very important role, which has been understated very often. Current changes in agriculture, in socio-economics environment, and an increasing urban population of consumers, make it difficult to forecast how maize diversity will evolve in the future. Consumers could prefer cheap and uniform maize products or could follow the path that can be discerned in Europe, where niche products, very often recognized by a geographic indication have reached niche markets. These niche markets could be promoted through the recognition of maize as a part of a cultural patrimony. Ortega (2003, p. 153) is also proposing such a strategy. These actions could be part of a governmental program aiming to re-vitalize rural areas in the country. Other factors that affect local and indigenous populations should be taken into account when formulating national or international policy issues. Agricultural subsidies in developed countries coupled with free trade

agreements may affect the economic viability of smallholder farmers in developing countries. Migration of farmers outside their region has also complex consequences, including on crop diversity, but its implication on maize diversity is beyond the scope and beyond the expertise of the authors of this chapter.

The introduction of transgenes in the Mexican agriculture will add more complexity. We have shown in this chapter that it is very difficult to evaluate *ex ante* all the possible consequences of the introduction of transgenes in Mexican maize crop. The difficulties come from the specificity of Mexican agriculture, which makes it difficult to directly use the information produced outside this country on transgene effects (Bellon and Berthaud, 2004). The difficulties come also from the fact that genetic engineering is still a new technology with still many unknown, characterized by a lack of control over the site of insertion and subsequent expression of transgenes. The complexity is also due to an evolution which relies on more than just biological factors. We have shown that farmers have a direct impact on the evolution of diversity through decisions they take based on socio-economical and cultural grounds. The adventitious presence of some specific transgenes (i.e., genes producing pharmaceutical compounds) may trigger a rejection of local landraces perceived as contaminated by farmers and consumers.

Many of the authors of chapters in this report agree that maize diversity is at risk in Mexico. The current agriculture system makes Mexico a center of diversity. Changes to this system will lower this diversity in many cases. Conservation of this reservoir of genetic resources for humanity will have to rely not only on biological rules but will have to mobilize a consolidated group of actors, from farmers to consumers, from NGO to the scientific community.

Lastly, as we have alluded to during this chapter, there are several unmet research needs that would provide essential data to fully assess the effect of gene flow (with or without transgenes) on maize and teosinte genetic diversity as well as the specific effect of transgenes on this diversity. We propose the following studies in the general area of flower biology, gene flow, and reproductive isolation of maize:

a) Documentation of the extent of the distribution of transgenes among maize landraces and teosinte populations need to be conducted or made public if these have been conducted.

b) Direct (cross-pollination frequency) and indirect (F_{ST} parameter) measurements of gene flow among maize landraces and cultivars and between maize and teosinte in different environments in Mexico.

c) Frequency of the *Ga1*, *Tcb1*, and any other potential crossability genes in different teosinte and landrace populations.

d) Fate of outcrossing events in subsequent generations of hybrids, in particular to determine whether there is any introgression into teosinte.

e) Analysis of transformation events at the molecular level and changes in structural and functional aspects following the introduction of transgenic "events" in new genetic backgrounds.

f) Role of farmers in selection and seed and pollen flows, including those with transgenes.

In addition, we believe that research conducted in this area should follow specific research protocols that include at least the following elements:

a) Guarantees about the availability of research methodology and results to public scrutiny following the completion of the experimentation phase and a peer-reviewed process.

b) Utilization of broad-based external advisory committees

c) Utilization of blind tests to eliminate potential biases

d) Guarantees as to the anonymity of specific farmers and locations, if deemed necessary.

We expect that other suggestions for research and research protocols will be made in other chapters and at the Oaxaca conference.

Glossary

Acriollado refers to an introduced commercial cultivar that has gone through the process of *creolization*.

Allele is a copy of a gene. Each individual has two copies of a gene, one inherited from the maternal parent and the other from the paternal parent.

Chloroplasts are organelles responsible for photosynthesis in plants. They contain their own genome consisting of DNA distinct from the nuclear genome.

Creolization is the gradual genetic modification of an introduced commercial cultivar through *hybridization* with local landraces.

Cytoplasm is the material surrounding the nucleus of a cell. It contains *organelles* (such as *chloroplasts* and *mitochondria*), membranes, and protein synthesis machinery.

Drift see *Genetic drift*

Ecological amplitude is the range of environmental conditions in which an organism is encountered.

F_1 is the first generation following *hybridization*.

Gamete is a reproductive cell. Female and male gametes unite to form a single cell called the zygote, which, through division, generates an embryo and ultimately a progeny individual.

Gene flow is the movement of genes from one population to another by way of *hybridization* of individuals in the two populations. In plants, gene flow can take place by pollen or seeds.

Gene silencing is the interruption or suppression of the expression of a gene at transcriptional or translational levels. A gene is sometimes silenced by the introduction of additional copies of that gene by genetic engineering.

Genetic assimilation is the displacement of genetic diversity of a population by high levels of *gene flow* into that population.

Genetic background. The genome of a plant comprises tens of thousands of genes. The genetic background is all the genes in the recipient plant. When an allele or a gene is moved from one plant to another through crossing and selection, the expression of the allele or gene that has moved can be modified considerably through interaction with these genes.

Genetic drift refers to random changes in *allele* frequencies due to chance events related to small population size and meiotic segregation.

Homozygosity is the situation in which the two *alleles* of a gene in an individual are the same.

Heterozygosity is the situation in which the two *alleles* of a gene in an individual are different.

Hitchhiking, see *Selective sweep*.

Hybridization is the crossing of two individuals.

Introgression is the introduction of a limited number of genes from a donor parent through *hybridization* followed by repeated hybridizations to the recipient parent (the introgressed parent).

Linkage refers to the probability that alleles of two genes, located closely on a chromosome, pass together to the next generation. Independent genes are genes situated on different chromosomes or far enough apart on the same chromosome to enable their alleles to be inherited independently (linkage = 0).

Linkage disequilibrium is the non-random association of *alleles* of different genes.

Marker is a DNA sequence, gene, or trait that is simply inherited; it behaves as a single gene and is not subject to environmental influences on its expression.

Migration is the movement of individuals or *gametes* between otherwise spatially isolated populations.

Migration-drift balance is an evolutionary situation in which *allele* frequencies in population are mainly controlled by either *migration* or *drift*.

Migration-selection balance is an evolutionary situation in which *allele* frequencies in population are mainly controlled by either *migration* or *selection*.

Mitochondria (sing. *Mitochondrion*) are *organelles* involved in the production of cellular energy. They contain their own genome consisting of DNA, distinct from the nuclear genome located within the nucleus.

Neutral marker is a *marker* that is not subject to *selection*, i.e. it does not affect the fitness of individuals positively or negatively.

Neutral diversity is the sum of genetic diversity that is not subject to selection, i.e. it does not affect the fitness of individuals positively or negatively.

Organelle is a subcellular structure located in the cytoplasm of cells. *Mitochondria* and *chloroplasts* are examples of organelles.

Recombination is the process by which alleles are exchanged between pairs of chromosomes (those inherited from the maternal and paternal parents) during sexual reproduction. Recombination creates new combinations of alleles at different loci along the chromosome.

Selective sweep refers to the reduction in genetic diversity in the chromosome region around a gene that is subject to positive selection.

Selection is the process whereby different individuals contribute different numbers of progenies to the next generation (through contrasting survival, vigor, or fertility). Individuals that exhibit higher fitness or adaptation to local conditions will contribute more individuals to the next generation and, thus, are said to be positively selected. Conversely, individuals that have lower fitness or adaptation will contribute fewer or no progeny to the next generation and are said to be negatively selected. Thus, the genes carried by positively selected individuals will gradually increase in frequency, whereas as the frequency of genes from negatively selected individuals will decrease.

References cited

Aguirre Gómez, J. A. 1999. Análisis regional de la diversidad del maíz en el Sureste de Guanajuato. Ph.D. thesis, México, D.F.: Universidad Nacional Autónoma de México, Mexico.

Alvarez-Morales, A. 2002. Transgenes in maize landraces in Oaxaca: Official report on the extent and implications [Online]. Available from Proceedings of the 7th International Symposium on the Biosafety of Genetically Modified Organisms Beijing, October 10-16, 2002 (p. 65-65) http://www.bba.de/gentech/isbgmo.pdf (verified February 16, 2004).

Anderson, E. 1947. Field studies of Guatemalan maize. Ann. Missouri Bot. Garden 34: 433-467.

Anderson, E., Cutler, H. 1942. Races of *Zea mays*: I. Their recognition and classification. Ann. Missouri Bot. Garden 21: 69-88.

Arias, L., L.Chávez, L. Latourniere, J. Cob, J. Canul, L. Burgos, E Sauri, R. Ortega, P. Ramírez, D. Williams y D. Jarvis. 2002. Diversidad de maíces de la milpa en Yaxcabá, Yucatán. In: Manejo de la diversidad cultivada en los agroecosistemas tradicionales. Resúmenes del Simposio 13-16 de febrero del 2002, Mérida, México. pp. 8 y 9

Badstue, L., Bellon, M.R., Berthaud, J., Ramírez, A., Flores, D., Juárez, X., ,. 2003. The dynamics of seed flow among small scale maize farmers in the Central Valleys of Oaxaca, Mexico. Paper presented at the International workshop on property rights collective action and local conservation of genetic resources. Rome Italy, September 29-October 2, 2003. http://www.capri.cgiar.org/pdf/bellon.pdf

Bellon, M.R. 2001. Participatory Research Methods for Technology Evaluation: A Manual for Scientists Working with Farmers. Mexico, D.F.: CIMMYT.

Bellon, MR, 2004. Conceptualizing intervention to support on-farm genetic resources conservation. World. Dev. 32:159-172

Bellon, M.R., and S. B. Brush, (1994). Keepers of maize in Chiapas, Mexico. Econ. Bot. 48: 196-209.

Bellon, M. R., Risopoulos, J. 2001. Small-scale farmers expand the benefits of improved maize germplasm: a case study from Chiapas, Mexico. World Dev. 29: 799-811.

Bellon, M.R., Berthaud, J., Smale, M., Aguirre, A.J., Taba, S., Aragón, F., Díaz, J., Castro, H. 2003a. Participatory landrace selection for on farm conservation: An example from the Central Valleys of Oaxaca, Mexico. Genetic Resources and Crop Evolution 50: 401-416.

Bellon, M. R., Adato, M., Becerril, J., Mindek, D. 2003b. The impact of improved maize germplasm on poverty alleviation: the case of Tuxpeño-derived material in Mexico, FCND Discussion Paper 162. IFPRI: Washington, DC.

Bellon, MR., Berthaud, J. 2004. Transgenic maize and the evolution of landrace diversity in Mexico. The importance of farmers'behavior. Plant Physiol. 134: 883-888.

Bennett, M., and I. Leitch. 1995. Nuclear DNA amounts in angiosperms. Ann. Bot. 76:113-176.

Bennetzen, J., 2000. Transposable element contributions to plant gene and genome evolution.. Plant Mol. Biol. 42: 251-269.

Berthaud, J., Pressoir, G., Ramírez-Corona, F., Bellon, M.R. 2002. Farmers' management of maize landrace diversity. A case study in Oaxaca and beyond. Available from Proceedings of the 7th International Symposium on the Biosafety of Genetically Modified Organisms Beijing, October 10-16, 2002 (pp. 79-88). International Biosafety Research Society http://www.bba.de/gentech/isbgmo.pdf

Blancas, L., Arias, D.M., Ellstrand, N.C. 2002. Patterns of genetic diversity in sympatric and allopatric populations of maize and its wild relative teosinte in Mexico: evidence for hybridization in Proceedings of « Ecological and Agronomic Consequences of Gene Flow from Transgenic Crops to Wild Relatives » Ohio State University, Columbus, OH, March 5-6, 2002

Bretting, P. K., Goodman, M.M. 1989. Karyotypic variation in Mesoamerican races of maize and its systematic significance. Econ. Bot. 43: 107-124.

Burke, J.M., and L.H. Rieseberg. 2003. Fitness effects of transgenic disease resistance in sunflowers. Science 300:1250-1250.

Charlesworth, B., M. Morgan, and D. Charlesworth. 1993. The effects of deleterious mutations on neutral molecular variation. Genetics 134:1289-1303.

Christou, P. 2002. No credible scientific evidence is presented to support claims that transgenic DNA was introgressed into traditional maize landraces in Oaxaca, Mexico. Transgenic Res. 11: iii-v.

CIMMYT. 2002. Two-tiered screens of maize lanfraces in CIMMYT gene bank and candidate lanraces for the collection indicate no presence of transgenes [Online]. Available from CIMMYT http://www.cimmyt.org/whatiscimmyt/transgenic/tiered_17Oct02.pdf (verified January 26, 2004).

Clark, R.M., E. Linton, J. Messing, and J.F. Doebley. 2004. Pattern of diversity in the genomic region near the maize domestication gene *tb1*. Proc. Nat. Acad. Sci. USA 101:700-707.

Cleveland, D., and D. Soleri, (eds.) 2002. Farmers, scientists, and plant breeding: integrating knowledge and practice, pp. 1-338. CABI, Wallingford, Oxon, UK.

Comai, L. 2000. Genetic and epigenetic interactions in allopolyploid plants. Plant Molec. Biol. 43:387-399.

Crossa, J. 1989. Methodologies for estimating the sample size required for genetic conservation of outbreeding crops. Theor. Appl. Genet. 77:153-161.

Crow, J.F., and M. Kimura. 1970. An introduction to population genetics theory. Harper & Row, New York.

Doebley, J. 1983. A brief note on the rediscovery of Durango teosinte. Maize Coop. Genet. Newsl. 57:127-128

Doebley, J. 1990. Molecular evidence and the evolution of maize. Econ. Bot. 44 (3 supplement): 6-27.

Doebley, J., Goodman, M.M., Stuber, C.W. 1984. Isoenzymatic variation in *Zea* (*Gramineae*). Syst. Bot. 9: 203-218.

Doebley, J., Goodman, M.M., Stuber, C.W. 1987. Patterns of isozyme variation between maize and mexican annual teosinte. Econ. Bot. 41: 234-246.

Doebley, J., Sisco, P.H. 1989. On the origin of the maize male sterile cytoplasms: its completely unimportant, that's why it's so interesting. Maize Coop. Genet. Newsl. 63:108-109

Doebley, J., Stec, A. 1993. Inheritance of the morphological differences between maize and teosinte: comparison of results for two F2 populations. Genetics 134: 559-570.

Ellstrand, N. C., Prentice, H.C., Hancock, J.F. 1999. Gene flow and introgression from domesticated plants into their wild relatives. Ann. Rev. Ecol. Syst. 30: 539-563.

Enciso L., A. 2002. Confirma el INE la presencia de transgénicos en cultivos de Oaxaca, La Jornada, Mexico, DF. http://www.jornada.unam.mx/2002/ago02/020812/042n1soc.php?origen=soc-jus.html

Enciso L., A., and A.T. Morales. 2002. *Nature* se niega a publicar estudio sobre transgénicos, La Jornada, Mexico, D.F. http://www.jornada.unam.mx/2002/oct02/021022/014n2pol.php?origen=politica.html

ETC. 2003. Contamination by genetically modified maize in Mexico much worse than feared [Online]. Available from ETC http://www.etcgroup.org/documents/NR_Maize_10_03ENG3.pdf (verified January 26, 2004).

Evans, M.M.S., Kermicle, J.L. 2001. *Teosinte crossing barrier1*, a locus governing hybridization of teosinte with maize. Theor. Appl. Genet. 103:259-265.

Fagard, M., Vaucheret, H. 2000. (Trans)genes silencing in plants: how many mechanisms? Ann. Rev. Plant Phys. Plant Mol. Biol. 51: 167-194.

Gahakwa, D., Maqbool, S.B., Fu, X., Sudhakar, D., Christou, P., Kohli, A. 2000. Transgenic rice as a system to study the stability of transgene expression: multiple heterologous transgenes show similar behaviour in diverse genetic backgrounds. Theor. Appl. Genet. 101:388–399.

Gepts, P. 2004a. Domestication as a long-term selection experiment. Plant Breed. Rev. 24 (Part 2): 1-44.

Gepts, P. 2004b. Who owns biodiversity and how should the owners be compensated? Plant Phys. 134: 1295–1307.

Gepts, P., and R. Papa. 2003. Possible effects of (trans)gene flow from crops on the genetic diversity from landraces and wild relatives. Envir. Biosafety Res. 2:89-103.

Goodman, M.M., Brown, W.L. 1988. Races of corn. Pp. 33-79. In « Corn and corn improvement third edition », Sprague, G.F., Dudley, J.W. (eds.), Agronomy N°18 American Society of Agronomy Inc Publishers, Madison, Wisconsin, USA

Grandbastien, M-A. 1998. Activations of plant retrotransposons under stress conditions. Trends Plant Sci. 3: 181-187

Gutiérrez-Nava, M. L, Warren, C.A., León, P., Walbot, V. 1998. Transcriptionally Active *MuDR*, the regulatory element of the Mutator transposable element family of *Zea mays*, is present in some accessions of the Mexican land race Zapalote chico. Genetics 1998 149: 329-346

Guzmán, R. 1978. Una nueva localidad para el teosinte *Zea perennis* y primer reporte de *Zea mexicana* para Jalisco. Bol. Inf.Inst. Bot. Univ. de Guadalajara 6:9-10

Haygood, R., A.R. Ives, and D.A. Andow. 2003. Consequences of recurrent gene flow from crops to wild relatives. Proc. Roy. Soc. London - Series B: Biol. Sci. 270:1879-1886.

Iltis, H.H., Benz, B.F., 2000. *Zea nicaraguensis* (Poaceae), a New Teosinte from Pacific Coastal Nicaragua. Novon: Vol. 10, No. 4. pp. 382–390

Iltis, H.H., Doebley, J.F. 1980. Taxonomy of *Zea* (*Gramineae*) II. Sub-specific categories in the *Zea mays* complex and a generic synopsis. Amer. J. Bot.. 67 :994-1004.

Iltis, H.H., Doebley, J.F., Guzmán, R., Pazy, B. 1979. *Zea diploperennis* (Gramineae): A new teosinte from Mexico. Science 303:186-188.

Iyer, L.M., S.P. Kumpatla, M.B. Chandrasekharan, and T.C. Hall. 2000. Transgene silencing in monocots. Plant Mol. Biol. 43:323-346.

Jenuwein, T., and C.D. Allis. 2001. Translating the histone code. Science 293:1074-1080.

Kato, Y.T.A. 1984. Chromosome morphology and the origin of maize and its races. *in* Evolutionary Biology Vol. 17 (Eds. M.K. Hecht, B. Wallace and G.T. Prance), Plenum, New York

Kermicle, J.L., Allen, J.O. 1990. Cross-incompatibility between maize and teosinte. Maydica 35: 399-408.

Lenormand, T. 2002. Gene flow and the limits to natural selection. Trends Ecol. Evol. 17:183-189.

Linder, C.R., Taha, I., Seiler, G.J., Snow, A.A., Rieseberg, L.H. 1998. Long-term introgression of crop genes into wild sunflower populations. Theor. Appl. Genet. 96: 339-347

Louette, D., Charrier, A., Berthaud, J. 1997. *In situ* conservation of maize in Mexico: Genetic diversity and maize seed management in a traditional community. Econ. Bot. 51: 20–38.

Louette, D., Smale, M. 2000. Farmers' seed selection practices and traditional maize varieties in Cuzalapa, Mexico. Euphytica 13: 25-41.

Mangelsdorf, PC. 1985. Teosinte introgression, a probable source of mobile genetic elements MNL 59:26-28

Martínez-Soriano J.P.R., Leal-Klevezas DS. 2000. Transgenic maize in Mexico: No need for concern. Science 287: 1399.

Matsuoka, Y., Y. Vigouroux, M. M. Goodman, J. Sanchez G., E. Buckler, and J. Doebley (2002). "A single domestication for maize shown by multilocus microsatellite genotyping," *Proceedings of the National Academy of Sciences* 99: 6080-6084.

Morris, M. L., Risopoulos, J., Beck, D.1999a. Genetic change in farmer-recycled maize seed: a review of the evidence. CIMMYT Economics Working Paper 99-07. Mexico, D.F.: CIMMYT.

Morris, M., Lopez-Pereira, M.A., 1999b. Impacts of Maize Breeding Research in Latin America 1966-1997. Mexico, D.F.: CIMMYT.

Mugo, S.N., D. Bergvinson, and D. Hoisington. 2001. Options in developing stemborer-resistant maize: CIMMYT's approaches and experiences. Insect Sci. Appl. 21:409-415.

Ortega-Paczka, R. 1973. Variación en maíz y cambios socioeconómicos en Chiapas México 1946-1971. Tesis de M.C. Colegio de Postgraduados, Rama de Botánica. Chapingo, México. 200p.

Ortega- Paczka, R. 2002. Proyectos de conservación *in situ* y mejoramiento participativo de maíz en México. Rev. Geogr. Agrícola 31: 97-108.

Ortega- Paczka, R. 2003. La diversidad del maíz en México. In G. Esteva and C. Marielle, coordinators, pp. 123-154. Mexico, DF: Ed. CONACULTA/ Museo Nacional de Culturas Populares.

Padgette, S.R., K.H. Kolacz, X. Delannay, D.B. Re, B.J. Lavallee, C.N. Tinius, W.K. Rhodes, Y.I. Otero, G.F. Barry, D.A. Eichholtz, V.M. Peschke, D.L. Nida, N.B. Taylor, and G.M. Kishore. 1995. Development, identification, and characterization of a glyphosate-tolerant soybean line. Crop Sci. 35:1451-1461.

Papa, R., and P. Gepts. 2003. Asymmetry of gene flow and differential geographical structure of molecular diversity in wild and domesticated common bean (*Phaseolus vulgaris* L.) from Mesoamerica. Theor. Appl. Genet. 106:239-250.

Pawlowski, W.P., and D.A. Somers. 1998. Transgenic DNA integrated into the oat genome is frequently interspersed by host DNA. Proc. Nat. Acad. Sci. USA 95:12106-12110.

Penagos, D., R. Magallanes, J. Valle, J. Cisneros, A. Martínez, D. Goulson, J. Chapman, P. Caballero, R. Cave, and T. Williams. 2003. Effects of weeds on insect pests of maize and their natural enemies in Southern Mexico. Intern. J. Pest Manag. 49:155-161.

Perales, H., Brush, S.B., Qualset, C.O., 2003a. Landraces of maize in Central Mexico : an altitudinal transect. Econ. Bot. 57: 7-20.

Perales, H., Brush, S.B., Qualset, C.O., 2003b. Dynamic management of maize landraces in Central Mexico. Econ. Bot. 57: 21-34.

Pressoir, G., Berthaud, J. 2004a. Patterns of population structure in maize landraces from the Central Valleys of Oaxaca in Mexico Heredity 92: 88-94.

Pressoir, G., Berthaud, J. 2004b. Population structure and strong divergent selection shape phenotypic diversification in maize landraces. Heredity 92: 95 – 101.

Quist, D., and I.H. Chapela. 2001. Transgenic DNA introgressed into traditional maize landraces in Oaxaca, Mexico. Nature 414: 541-543.

Quist, D., and I.H. Chapela. 2002. Biodiversity (Communications arising (reply): Suspect evidence of transgenic contamination/Maize transgene results in Mexico are artefacts. Nature 416: 602-602.

Remington, D.L., J.M. Thornsberry, Y. Matsuoka, L.M. Wilson, S.R. Whitt, J. Doeblay, S. Kresovich, M.M. Goodman, and E.S. Buckler. 2001. Structure of linkage disequilibrium and phenotypic associations in the maize genome. Proc. Nat. Acad. Sci. USA 98:11479-11484.

Sánchez G, J. J., Goodman, M.M., Stuber, C.W. 2000b. Isoenzymatic and morphological diversity in the races of maize in Mexico, Econ. Bot. 54: 43-59.

Sánchez G, J. J., Stuber, C.W., Goodman, M.M. 2000a. Isoenzymatic diversity in the races of maize in the Americas, Maydica 45: 185-203.

Sánchez G, J.J., Ordaz, S.L. 1987. El teocintle in Mexico. Systematics and Ecogeographical Studies of Genepools n°2, 50 pp.

Sánchez-G, J.J., Kato-Y, T.A., Aguilar-S, M., Hernandez-C., J.M., Lopez-R, A., Ruiz-C, J.A.1998. Distribubución y caracterización del teocintle. Libro técnico No 2. CIPAC-INIFAP-SAGAR, Guadalajara, Jalisco,Mexico

Serratos, J.A., Wilcox, M., Castillo, F., (eds.) 1996. Flujo genético entre maíz criollo, maíz mejorado y teocintle: Implicaciones para el maíz transgénico. Mexico D.F. CIMMYT

Smale, M., Aguirre, A., Bellon, M.R., Mendoza, J., Manuel Rosas, I. 1999. Farmer management of maize diversity in the Central Valleys of Oaxaca, Mexico: CIMMYT/INIFAP 1998 Baseline Socioeconomic Survey. CIMMYT Economics Working Paper 99-09. Mexico, D.F.: CIMMYT.

Smith, J.S.C., Goodman, M.M., Stuber, C.W. 1984. Variation within teosinte. III. Numerical analysis of allozyme data. Econ. Bot. 38: 97-113.

Smith, J.S.C., Goodman, M.M., Stuber, C.W. 1985. Relationships between maize and teosinte of Mexico and Guatemala: numerical analysis of allozyme data. Econ. Bot. 39: 12-24.

Smith, M.E., J.A. Mihm, and D.C. Jewell. 1987. Breeding for multiple resistance to temperate, subtropical, and tropical maize insect pests at CIMMYT, p. 222-234 Toward insect resistant maize for the Third World: Proceeding of the International Symposium on Methodologies for Developing Host Plant Resistance to Maize Insects. CIMMYT, Mexico, DF.

Snow, A., D. Pilson, L. Rieseberg, M. Paulsen, N. Pleskac, M. Reagon, D. Wolf, and S. Selboa. 2003. A Bt transgene reduces herbivory and enhances fecundity in wild sunflowers. Ecol. Applic. 13: 279–286.

Snow, A.A., Moran-Palma, P., Rieseberg, L.H., Wszelaki, A., Seiler, G.J. 1998. Fecundity, phenology, and seed dormancy of F_1 wild-crop hybrids in sunflower (*Helianthus annuus*, Asteraceae). Amer. J. Bot. 794-801.

Svitashev, S., E. Ananiev, W.P. Pawlowski, and D.A. Somers. 2000. Association of transgene integration sites with chromosome rearrangements in hexaploid oat. Theor. Appl. Genet. 100: 872-880.

Svitashev, S.K., W.P. Pawlowski, I. Makarevitch, D.W. Plank, and D.A. Somers. Complex transgene locus structures implicate multiple mechanisms for plant transgene rearrangement. Plant J. 32: 433-445.

van Driel, R., P.F. Fransz, and P.J. Verschure. 2003. The eukaryotic genome: a system regulated at different hierarchical levels. J. Cell Sci. 116:4067-4075.

Vain, P., James, V.A., Worland, B., Snape, J.W. 2002. Transgene behaviour across two generations in a large random population of transgenic rice plants produced by particle bombardment. Theor Appl Genet. 105:878-889.

Wang, R.-L., A. Stec, J. Hey, L. Lukens, and J. Doebley. 1999. The limits of selection during maize domestication. Nature 398: 236-239.

Wang, R.-L., A. Stec, J. Hey, L. Lukens, and J. Doebley. 2001. correction: The limits of selection during maize domestication. Nature 410: 718.

Wang, T., Li, Y., Shi, Y., Reboud, X., Darmency, H., Gressel, J. 2004. Low frequency transmission of a plastid-encoded trait in *Setaria italica* . Theor. Appl. Genet. 108 (2): 315-320Weissinger, A.K., Timothy, D.H., Levings III, C.S., Goodman, M.M. 1983. Patterns of mitochondrial DNA variation in indigenous maize races of Latin America. Genetics 104 : 365-379

Wellhausen, E. J., Roberts, L.M., Hernández Xolocotzi, E., Mangelsdorf, P.C. 1952. Races of Maize in México: Their Origin, Characteristics, and Distribution. Cambridge, MA: The Bussey Institution, Harvard University. 223 p.

Whitton, J., Wolf, D.E., Arias, D.M., Snow, A.A., Rieseberg, L.H., 1997. The persistence of cultivar alleles in wild populations of sunflowers five generations after hybridization, Theor. Appl. Genet. 95: 33-40.

Wilkes, H.G., 1967. Teosinte: the closest relative of maize. Ph.D. dissertation. Bussey Inst. Harvard Univ. 159p.

Windels, P., I. Taverniers, A. Depicker, E. Van Bockstaele, and M. De Loose. 2001. Characterisation of the Roundup Ready soybean insert. Eur. Food Res. Technol. 213:107-112.

Yupit Moo, E. 2002. Seed storage systems of milpa crops and their pests in storage in Yaxcaba, Yucatan. In: Managing crop diversity in traditional agroecosystems. Proceedings of a Symposium, 13-16 February2002, Merida, Mexico. International Plant Genetic Resources Institute, Rome, Italy. p 43-44

Zhong, G.-Y. 2001. Genetic issues and pitfalls in transgenic plant breeding. Euphytica 118:137-144.