

## When Transgenes Wander, Should We Worry?

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It is hard to ignore the ongoing, often emotional, public discussion of the impacts of the products of crop biotechnology. At one extreme of the hype is self-righteous panic, and at the other is smug optimism. While the controversy plays out in the press, dozens of scientific workshops, symposia, and other meetings have been held to take a hard and thoughtful look at potential risks of transgenic crops. Overshadowed by the loud and contentious voices, a set of straightforward, scientifically based concerns have evolved, dictating a cautious approach for creating the best choices for agriculture's future.

Plant ecologists and population geneticists have looked to problems associated with traditionally improved crops to anticipate possible risks of transgenic crops. Those that have been most widely discussed are: (a) crop-to-wild hybridization resulting in the evolution of increased weediness in wild relatives, (b) evolution of pests that are resistant to new strategies for their control, and (c) the impacts on nontarget species in associated ecosystems (such as the unintentional poisoning of beneficial insects; Snow and Palma, 1997; Hails, 2000).

Exploring each of these in detail would take a book, and such books exist (e.g. Rissler and Mellon, 1996; Scientists' Working Group on Biosafety, 1998). However, let us consider the questions that have dominated my research over the last decade to examine how concerns regarding engineered crops have evolved. Those questions are: How likely is it that transgenes will move into and establish in natural populations? And if transgenes do move into wild populations, is there any cause for concern? It turns out that experience and experiments with traditional crops provide a tremendous amount of information for answering these questions.

The possibility of transgene flow from engineered crops to their wild relatives with undesirable consequences was independently recognized by several scientists (e.g. Colwell et al., 1985; Ellstrand, 1988; Dale, 1992). Among the first to publish the idea were two Calgene scientists, writing: "The sexual transfer of genes to weedy species to create a more persistent weed is probably the greatest environmental risk of planting a new variety of crop species" (Goodman and Newell, 1985). The movement of unwanted crop genes into the environment may pose more of a management dilemma than unwanted chemicals. A single molecule of DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] remains a single molecule or degrades, but a single crop allele has the opportunity

to multiply itself repeatedly through reproduction, which can frustrate attempts at containment.

In the early 1990s, the general view was that hybridization between crops and their wild relatives occurred infrequently, even when they were growing in close proximity. This view was supported by the belief that the discrete evolutionary pathways of domesticated crops and their wild relatives would lead to increased reproductive isolation and was supported by challenges breeders sometimes have in obtaining crop-wild hybrids. Thus, my research group set out to measure spontaneous hybridization between wild radish (*Raphanus sativus*), an important California weed, and cultivated radish (the same species), an important California crop (Klinger et al., 1991). We grew the crop as if we were multiplying commercial seed and surrounded it with stands of weeds at varying distances. When the plants flowered, pollinators did their job. We harvested seeds from the weeds for progeny testing. We exploited an allozyme allele (*Lap-6*) that was present in the crop and absent in the weed to detect hybrids in the progeny of the weed. We found that every weed seed analyzed at the shortest distance (1 m) was sired by the crop and that a low level of hybridization was detected at the greatest distance (1 km). It was clear, at least in this system, that crop alleles could enter natural populations.

But could they persist? The general view at that time was that hybrids of crops and weeds would always be handicapped by crop characteristics that are agronomically favorable, but a detriment in the wild. We tested that view by comparing the fitness of the hybrids created in our first experiment with their non-hybrid siblings (Klinger and Ellstrand, 1994). We grew them side by side under field conditions. The hybrids exhibited the huge swollen root characteristic of the crop; the pure wild plants did not. The two groups did not differ significantly in germination, survival, or ability for their pollen to sire seed. However, the hybrids set about 15% more seed than the wild plants. In this system, hybrid vigor would accelerate the spread crop alleles in a natural population.

When I took these results on the road, I was challenged by those who questioned the generality of the results. Isn't radish probably an exception? Radish is outcrossing and insect pollinated. Its wild relative is the same species. What about a more important crop? What about a more important weed? We decided to address all of those criticisms with a new system.

Sorghum (*Sorghum bicolor*) is one of the world's most important crops. Johnsongrass (*Sorghum halepense*) is one of the world's worst weeds. The two are distinct species, even differing in chromosome number, and sorghum is largely selfing and wind pollinated. Sorghum was about as different from radish as you could get.

We conducted experiments with sorghum paralleling those with radish. We found that sorghum and johnsongrass spontaneously hybridize, although at rates lower than the radish system, and detected crop alleles in seed set by wild plants growing 100 m from the crop (Arriola and Ellstrand, 1996). The fitness of the hybrids was not significantly different from their wild siblings (Arriola and Ellstrand, 1997). The results from our sorghum-johnsongrass experiments were qualitatively the same as those from our cultivated radish-wild radish experiments. Other labs have conducted similar experiments on crops such as sunflower (*Helianthus annuus*), rice (*Oryza sativa*), canola (*Brassica napus*), and pearl millet (*Pennisetum glaveum*; for review, see Ellstrand et al., 1999). In addition, descriptive studies have repeatedly found crop-specific alleles in wild relatives when the two grow in proximity (for review, see Ellstrand et al., 1999). The data from such experiments and descriptive studies provide ample evidence that spontaneous hybridization with wild relatives appears to be a general feature of most of the world's important crops, from raspberries (*Rubus idaeus*) to mushrooms (*Agaricus bisporus*; compare with Ellstrand et al., 1999).

When I gave seminars on the results of these experiments, I was met by a new question: "If gene flow from crops to their wild relatives was a problem, wouldn't it already have occurred in traditional systems?" A good question. I conducted a thorough literature review to find out what was known about the consequences of natural hybridization between the world's most important crops and their wild relatives.

Crop-to-weed gene flow has created hardship through the appearance of new or more difficult weeds. Hybridization with wild relatives has been implicated in the evolution of more aggressive weeds for seven of the world's 13 most important crops (Ellstrand et al., 1999). It is notable that hybridization between sea beet (*Beta vulgaris* subsp. *maritima*) and sugar beet (*B. vulgaris* subsp. *vulgaris*) has resulted in a new weed that has devastated Europe's sugar production (Parker and Bartsch, 1996).

Crop-to-wild gene flow can create another problem. Hybridization between a common species and a rare one can, under the appropriate conditions, send the rare species to extinction in a few generations (e.g. Ellstrand and Elam, 1993; Huxel, 1999; Wolf et al., in press). There are several cases in which hybridization between a crop and its wild relatives has increased the extinction risk for the wild taxon (e.g.

Small, 1984). The role of hybridization in the extinction of a wild subspecies of rice has been especially well documented (Kiang et al., 1979). It is clear that gene flow from crops to wild relatives has, on occasion, had undesirable consequences.

Are transgenic crops likely to be different from traditionally improved crops? No, and that is not necessarily good news. It is clear that the probability of problems due to gene flow from any individual cultivar is extremely low, but when those problems are realized, they can be doozies. Whether transgenic crops are more or less likely to create gene flow problems will depend in part on their phenotypes. The majority of the "first generation" transgenic crops have phenotypes that are apt to give a weed a fitness boost, such as herbicide resistance or pest resistance. Although a fitness boost in itself may not lead to increased weediness, scientists engineering crops with such phenotypes should be mindful that those phenotypes might have unwanted effects in natural populations. In fact, I am aware of at least three cases in which scientists decided not to engineer certain traits into certain crops because of such concerns.

The crops most likely to increase extinction risk by gene flow are those that are planted in new locations that bring them into the vicinity of wild relatives, thereby increasing the hybridization rate because of proximity. For example, one can imagine a new variety that has increased salinity tolerance that can now be planted within the range of an endangered relative. It is clear that those scientists creating and releasing new crops, transgenic or otherwise, can use the possibility of gene flow to make choices about how to create the best possible products.

It is interesting that little has been written regarding the possible downsides of within-crop gene flow involving transgenic plants. Yet a couple of recent incidents suggest that crop-to-crop gene flow may result in greater risks than crop-to-wild gene flow. The first is a report of triple herbicide resistance in canola in Alberta, Canada (MacArthur, 2000). Volunteer canola plants were found to be resistant to the herbicides Roundup (Monsanto, St. Louis), Liberty (Aventis, Crop Science, Research Triangle Park, NC), and Pursuit (BASF, Research Triangle Park, NC). It is clear that two different hybridization events were necessary to account for these genotypes. It is interesting that the alleles for resistance to Roundup and Liberty are transgenes, but the allele for Pursuit resistance is the result of mutation breeding. Although these volunteers can be managed with other herbicides, this report is significant because, if correct, it illustrates that gene flow into wild plants is not the only avenue for the evolution of plants that are increasingly difficult to manage.

The second incident is a report of the Starlink Cry9C allele (the one creating the fuss in Taco Bell's taco shells) appearing in a variety of supposedly

nonengineered corn (Callahan, 2000). Although unintentional mixing of seeds during transport or storage may explain the contamination of the traditional variety, inter-variety crossing between seed production fields could be just as likely. This news is significant because, if correct, it illustrates how easy it is to lose track of transgenes. Without careful checking, there are plenty of opportunities for them to move from variety to variety. The field release of "third generation" transgenic crops that are grown to produce pharmaceutical and other industrial biochemicals will pose special challenges for containment if we do not want those chemicals appearing in the human food supply.

The products of plant improvement are not absolutely safe, and we cannot expect transgenic crops to be absolutely safe either. Recognition of that fact suggests that creating something just because we are now able to do so is an inadequate reason for embracing a new technology. If we have advanced tools for creating novel agricultural products, we should use the advanced knowledge from ecology and population genetics as well as social sciences and humanities to make mindful choices about how to create the products that are best for humans and our environment.

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