Stakeholder Workshop
"Future Directions & Research Priorities for the
USDA Biotechnology Risk Assessment Grants Program"
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Research Needs & Priorities for Plants: Impact & Management of Gene Flow[1]

Research needs and priorities regarding the introgression of transgenes from crops to wild and feral relatives were discussed. Research over the past decade has amply demonstrated that the occurrence of gene flow from crops to wild species is greater than some had anticipated. Although we better appreciate the possibility of gene flow, some important factors affecting gene flow levels are still not well understood. The consequences of gene flow, particularly introgression of transgenic loci into wild populations, are less understood. Research gaps and priorities were identified as follows.

Risk Assessment

Gene flow from crops to wild populations, through pollen, has been amply demonstrated in the past decade. More work is needed to understand the factors controlling the rate and spatial dimensions associated with gene flow. Much less is known about the fate of crop genes entering wild populations. Specific points of importance follow.

<u>DETECTION OF GENE FLOW</u>

- How does regional variation in pollinator communities affect regional differences in pollen movement?
- Species of wild relatives are not genetically equivalent across their geographic ranges. Does the crop-relative crossing potential change with location? Does hybridization potential vary with crop variety?
- Avenues for gene flow, beyond pollen movement, need to be better characterized. For instance animal dispersal, seed spillage, seed dispersal, and mixture of seed can all move genes. Vegetative propagules (rhizomes, tubers, stolons, etc.) can also be moved by human and natural causes. How important are these for particular crop species systems?
- The potential for gene flow may differ depending on whether the insertion is made into the nuclear versus the cytoplasmic genomes. Although chloroplasts and mitochondria are primarily maternally inherited, 'leakage' has been observed in some systems. We need to know how common leakage may be and how it would affect introgression rates.
- Methods are needed for detecting long-distance pollen movement. Our consensus was that there is a need for selectively neutral, easily scoreable genetic markers that could be released for gene flow studies. Green Fluorescent Protein (GFP) transgenes are candidates. Such constructs could be labeled with neutral DNA sequences for unique identification. The regulatory issues associated with this approach deserve serious consideration.
- Use of non-transgenic, crop-specific markers (e.g., AFLP loci) can serve as indicators of long-distance flow in some cases, but without historical information, interpretation of geographic patterns is often problematic. Studies that have this baseline information are encouraged.

CONSEQUENCES OF GENE FLOW

- Do the phenotypic and fitness effects of transgenes change with genetic background (i.e., is the effect in the crop-wild hybrid predictable from the effect in the crop)?
- There is a general lack of knowledge of how transgenes affect fitness in the F_1 and its subsequent F_2 and back cross generations.
- Effects of transgenes on the population dynamics of wild or feral plants, their competitive ability, and impact on plant community structure need further investigation.
- Testable models for introgression of transgenes conferring adaptive traits (e.g., pest resistance, salt or drought tolerance) and domestication traits (e.g., dwarfism, male and female sterility) need to be developed. hese efforts must be accompanied by experimentation to determine reasonable parameter values.
- What is the variation in introgressive potential among insertion events for the same transgenic construct? How does this variation compare with introgression of genes modified by more conventional processes (such as mutagenesis)? This information could help determine the extent to which continued event-by-event regulation is warranted.

Taking the larger view, the group agreed that because the likelihood and consequences of gene flow is dependent on the contingencies of the particular cropping system, a thorough understanding of environmental risk requires a multidisciplinary approach. Studies at both the field and landscape levels, covering multiple generations (i.e., $F_{1,}$ F_2 and continued back crosses), or for multiple years for vegetatively-propagated crops, are needed to determine when a statistically significant effect is also an environmentally material effect. Such studies can also evaluate the risk/benefit structure of genetic modification to alternative technologies.

Risk Management

The addition of Risk Management to the scope of the USDA Biotechnology Risk Assessment Grants Program in fiscal year 2003 requires new research initiatives concerning topics such as confinement strategies.

BIOLOGICAL CONFINEMENT

- Continued development of biological confinement technologies, such as auto-suicidal mechanisms, tissue-specific excision, and chloroplast transformation, etc., should be supported in a wider range of crops. Studies that combine methods to provide redundant, but practical, systems for confinement are encouraged.
- Many of these technologies will have proprietary restrictions. The group recommends public financial support of this research when it will lead to improved understanding of their effectiveness in the environment, provided that grant recipients can offer reasonable public access to these technologies.
- Stability of biological confinement technologies requires scrutiny. Breakdown of constructs, selection for counter-acting modifiers, gene silencing, and leakage are potential failure mechanisms that should be investigated.

PHYSICAL CONFINEMENT

- Efficacy of 'trap crop' border rows needs further evaluation for designing high-level confinement plans in field tests. However, because of the limited funding for the program, studies that develop novel approaches of broad application should be favored over highly crop- or regional-specific studies.
- Physical confinement may be ineffective against long-distance pollen dispersal. Modeling approaches, including those modifying existing model structures from epidemiology and operations research, should be applied to this issue when they can be validated by convention or transgenic markers.

New molecular technologies to promote biological confinement are likely to arise. Quality research on the combined effects of biological and physical confinement should be supported.

Mitigation

Mitigation refers to traits that do not directly prevent gene flow and introgression, but would slow the spread or limit the expression of genes that are released. The group recognizes that for some traits, such as vertebrate toxins and many pharmaceuticals, no level of gene flow is acceptable, and mitigation is a moot point.

- The stacking of 'domestication' genes with transgenes, such as those that reduce competitiveness in the wild, can limit their introgression; the fitness penalty of certain domestication phenotypes would put crop-wild hybrids at a selective disadvantage. Further investigation on this point should be supported.
- Methods to minimize crop-to-crop gene flow to acceptable levels, especially in seed production areas, needs development.
- Mechanisms that down-regulate transgene expression in natural environments merit scrutiny.

Additional topics, such as remediation in the case of an adverse event, were mentioned, but did not stimulate discussion about possible research areas.

Monitoring

The most desirable monitoring protocols require reliable baseline information, including historical levels of variation over space and time. Extensive databases, unfortunately, will seldom be available. Nevertheless, several research areas were identified that can inform the design of monitoring programs.

- Confined field experiments in crop-wild relative systems, starting with reasonable baseline information, can serve as an important means to study the effectiveness of monitoring programs.
- Studies on the introgression of non-transgenic alleles from crop to wild relatives (e.g., a spontaneous mutant for herbicide resistance) sometimes can be used to guide monitoring procedures, and should be supported.
- Introgression from crops to wild relatives may be a rare event. There is great need for statistical modeling to develop sampling strategies with sufficient power to allow early detection of such events. Methodology developed for epidemiology and in operations research may be applicable to this purpose.

- As mentioned above, unique DNA markers, including transgenic methods, should be developed to assist with efforts to monitor certain types of transgenes.

The research establishing crop-to-wild relative gene flow in the past decade is a solid foundation for research on the consequences of gene flow. Many valuable insights on the proper design of monitoring programs will emerge as a by-product of such efforts.

Priorities

The group considered all of the above items worthy of research support. Following the conference, the group was consulted, and the following items emerged as priorities for funding.

Highest priorities

- Continued development of biological confinement technologies, such as auto-suicidal mechanisms, tissue-specific excision, and chloroplast transformation, etc., should be supported in a wider range of crops.
- Measuring impact of transgene placement (nuclear or cytoplasmic) on the flow and introgression of transgenes into wild and feral plants, especially as a means of confinement.

High priorities

- Determining how transgenes of various kinds (stress/pest resistance, domestication traits, etc.) affect fitness and competitive ability in the F_1 and its subsequent back cross generations, along with their population dynamics and community effects.
- Development of ecologically neutral markers to detect and measure long-distance pollen dispersal.

Priorities

- Modeling of important processes, such as long-distance gene flow and dynamics of hybrid populations, especially as such models can inform the sampling strategies of monitoring programs.
- Support for multidisciplinary, long term investigation of gene flow in cropping systems.

[1] Some of the research needs and priorities listed in this document may be outside the scope of the USDA Biotechnology Risk Assessment Grants Program. This document was prepared by one or more of the individuals listed below. USDA program staff did not edit the content of this document. The USDA Biotechnology Risk Assessment Grants Program supports risk assessment and risk management research projects regarding the safety of introducing into the environment genetically modified animals, plants, and microorganisms. More information is available at: www.reeusda.gov/crgam/biotechrisk/biotech.htm. Questions regarding the suitability of research proposals should be discussed with the Program Director (dhamernik@csrees.usda.gov).

A list of people that attended this workshop is available at: www.isb.vt.edu/brarg_meeting.htm . The following individuals contributed to the discussion of this topic at the workshop and/or preparation of this document after the workshop:

Representatives from U.S. Regulatory Agencies:

Bruce MacBryde (USDA-APHIS)
Chris Wozniak (EPA)

Discussion Leader:

Allison Snow (Ohio State University)

Reporters:

Carol Mallory-Smith (Oregon State University)
Art Weis (University of California, Irvine)

Science Facilitators:

Henry Daniell (University of Central Florida)
Linda Hall (Government of Alberta)
Michael Horak (Monsanto)
Steve Strauss (Oregon State University)