THE PLANNED INTRODUCTION OF GENETICALLY ENGINEERED ORGANISMS: ECOLOGICAL CONSIDERATIONS AND RECOMMENDATIONS[1]

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Abstract. The ecological and evolutionary aspects of planned introductions of transgenic organisms into the environment are considered in this report. The authors support the timely development of environmentally sound products, such as improved agricultural varieties, fertilizers, pest control agents, and microorganisms for waste treatment, through the use of advanced biotechnology within the context of a scientifically based regulatory policy that encourages innovation without compromising sound environmental management. Economic, social, and ethical concerns also must be weighed along with strictly ecological and evolutionary considerations, but these other issues are beyond the scope of this report.

Ecological oversight of planned introductions should be directed at promoting effectiveness while guarding against potential problems. The diversity of organisms that will be modified, functions that will be engineered, and environments that will receive altered organisms makes ecological risk evaluation complex. While we cannot now recommend the complete exemption of specific organisms or traits from regulatory oversight, we support and will continue to assist in the development of methods for scaling the level of oversight needed for individual cases according to objective, scientific criteria, with a goal of minimizing unnecessary regulatory burdens. In this report, we provide a preliminary set of specific criteria for the scaling of regulatory oversight.

Genetically engineered organisms should be evaluated and regulated according to their biological properties (phenotypes), rather than according to the genetic techniques used to produce them. Nonetheless, because many novel combinations of properties can be achieved only by molecular and cellular techniques, products of these techniques may often be subjected to greater scrutiny than the products of traditional techniques. Although the capability to produce precise genetic alterations increases confidence that unintended changes in the genome have not occurred, precise genetic characterization does not ensure that all ecologically important aspects of the phenotype can be predicted for the environments into which an organism will be introduced.

Many important scientific issues must be considered in evaluating the potential ecological consequences of the planned introduction of genetically engineered organisms into the environment. These include survival and reproduction of the introduced organism, interactions with other organisms in the environment, and effects of the introduced organism on ecosystem function. We encourage the use of small-scale field tests, when justified by previous laboratory and/or greenhouse studies, under conditions that minimize dispersal and under appropriate regulatory oversight. As the biotechnology industry develops, continuing regulatory oversight as well as long-term research and monitoring will be necessary for responsible risk management.

Many engineered organisms will probably be less fit than the parent organism, although some important exceptions may arise. Even if an engineered trait reduces an organism's fitness only slightly, many generations may pass before the introduced organism disappears completely due to decreased fitness. Such persistence is most probable when the turnover rate of populations is very slow.

Natural selection will act on genetically engineered organisms, as it does on all others. Selection after the release of the transgenic organism will tend to increase fitness, not

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decrease it, by reducing the costs associated with the novel traits. If increases in fitness do occur, they will probably increase population growth rate and biological competitiveness, or produce other ecological effects that should be considered in assessing risks.

Transfer of engineered genes from the modified organism to other organisms may occur through hybridization in higher organisms, or through conjugation, transduction, or transformation in microorganisms. If lateral transfer occurs, an engineered gene may persist in the natural environment even after the genetically engineered organism itself is no longer present. The available scientific evidence indicates that lateral transfer among microorganisms in nature is neither so rare that we can ignore its occurrence, nor so common that we can assume that barriers crossed by modern biotechnology are comparable to those constantly crossed in nature.

Native species, as well as species newly introduced from distant habitats, may become pests. An organism engineered to prosper in a new habitat type, geographic area, or season is effectively an introduced organism in that it will probably enter into new biotic and abiotic interactions. Therefore, regulatory and risk assessment structures that rely on the distinction between "native" and "non-native" must be used with caution.

Concern has frequently been expressed regarding the potential for genetically engineered organisms to displace resident species in the receiving community, particularly microbial species performing key functional roles such as nitrogen fixation or lignin decomposition. Because redundancy of function appears to be common in microbial communities, in many cases there would be little concern over microbial species displacement caused by an introduced transgenic organism.

Ecological effects and the geographic ranges of organisms transcend political boundaries; we therefore consider it essential to promote and achieve international coordination of risk assessment and regulation of biotechnology. Special consideration must be given to the protection of rare genetic resources, such as the wild ancestors of domesticated species, and threatened gene pools of other wild species. We urge local, state, national, and international cooperation in risk assessment and regulation of the ecological effects of the introduction of transgenic organisms.

Evaluating the benefits and risks of biotechnology products requires expertise in many scientific disciplines including molecular biology, genetics, cell biology, evolutionary biology, physiology, population and community ecology, and ecosystem science. For society to realize the full benefits of biotechnology, interdisciplinary research and graduate training programs are needed to expand the expertise of the scientific community at large.

INTRODUCTION

The ecological and evolutionary aspects of the planned environmental testing and large-scale use of genetically engineered organisms are considered in this report. New techniques of molecular and cellular biology, including recombinant DNA and cell fusion, have emerged as powerful research tools in biology. As the foundations of biotechnology, these techniques hold great promise for the development of new products derived from plants, animals and microorganisms to be employed in industry, agriculture, and health care.

Scientific issues involved in the introduction of genetically engineered organisms (also termed transgenic organisms) into the environment are addressed in this report. They include the potential effects of genetically engineered organisms on community and ecosystem processes, the effects of added genes on fitness, the potential for unintended transfer of genes to other species, the effects of scale and frequency of introductions, the applicability of several models for assessing the potential ecological consequences, and a proposal for the scaling of regulatory oversight.

The writers and reviewers of this report, most of whom are members of the Ecological Society of America, represent the wide spectrum of professional ecological and evolutionary expertise encompassed by the 6500 members of the Society. **Although we support the timely development of environmentally sound products through the use of advanced biotechnology, we believe that these developments should occur within the context of a scientifically based regulatory policy that encourages innovation without compromising sound environmental management.**

The issues involved in the environmental introduction of transgenic organisms are complex and interdisciplinary. The development of effective biotechnology products and the assessment of their benefits and risks require expertise from many disciplines, in both natural and social sciences. This report is limited in scope to consideration of the ecological and evolutionary aspects of planned environmental introduc-

tions of genetically engineered organisms. Economic, social, and ethical concerns also must be weighed along with strictly ecological and evolutionary considerations.

Molecular and cellular biotechnology (including recombinant DNA, electroporation, projectile insertion, nuclear microinjection, and cell fusion) offers the potential for reaching many of the same goals pursued by traditional methods of plant and animal breeding and by mutation and selection of microorganisms, but in a more precise and efficient manner. For example, plant breeders have long sought to increase the disease resistance of crops through selection of resistant varieties and by hybridizing crops with wild relatives (Mayo 1987). Molecular techniques now permit the direct and precise introduction of genes from wild relatives, and cellular methods allow screening for the desired phenotype to proceed more efficiently. In addition to allowing the more rapid and precise accomplishment of such traditional objectives, however, molecular techniques now permit the integration of genetic information from very distantly related organisms, producing genotypes that no previous technology could ever have produced (e.g., Jaynes et al. 1987, Wong et al. 1988).

Novelty of a genotype, in itself, is not remarkable: every human embryo, unless an identical twin, has a genotype new to the planet. The novelty of biotechnology is its ability to exploit the universality of the genetic code to combine, in a single organism, major adaptive traits developed by organisms that have evolved along separate phylogenies. Organisms with novel combinations of traits are more likely to play novel ecological roles, on average, than are organisms produced by recombining genetic information existing within a single evolutionary lineage.

One prominent example of the new ability to combine traits from unrelated organisms is illustrated by the incorporation of a gene for the endotoxin of the bacterium *Bacillus thuringiensis* (or "*Bt*"), a registered microbial insecticide, into the genome of crop plants to protect them from insect damage (Goodman et al. 1987). For example, even though a tomato plant with an inserted *Bt* gene is most definitely still a tomato plant, its properties—as an organism, as a component of an agricultural ecosystem, and as a producer of human food—warrant investigation, because they may or may not parallel the properties of plants produced by traditional breeding techniques.

In addition to the ability to move genes among distantly related organisms, molecular techniques permit the incorporation of DNA sequences that code for completely novel proteins designed by the experimenter. For example, entirely synthetic proteins have been designed to supplement the overall production of essential amino acids in potatoes. The appropriate DNA sequence is first designed and synthesized in the laboratory, then inserted into the potato DNA, along with sequences to regulate the expression of the new protein. This technique is already yielding transgenic potatoes of considerable promise for the alleviation of human malnutrition (Jaynes et al. 1986), but much work remains to be done with these transgenic varieties to assess their nutritional characteristics, pest and disease tolerance, role in agricultural ecosystems, and hazards of hybridization with wild relatives. Some of these assessments are routine for new varieties produced by traditional plant breeding, but others are genuinely new.

What ecology offers to the success of biotechnology

Professional ecologists are dedicated to the scientific study of interactions of organisms with one another and with the physical environment. Their work ranges from the study of the behavior and physiology of individual organisms in their environments, through the study of biological populations (single species) and interacting communities (many species), to the study of ecosystem function (biogeochemical transformations of energy and materials). The contributions that the field of ecology can offer biotechnology stem from the expertise derived from studying nature at these different levels of organization. A recent report published by the National Academy of Sciences details contributions made by the field of ecology to fisheries, wildlife, and forest management; pest control; and human health and welfare (National Research Council 1986*a*).

The National Academy of Sciences report, *Introduction of Recombinant DNA-Engineered Organisms Into the Environment: Key Issues* (1987), calls upon ecologists to provide guidance in evaluating planned introductions. Understanding ecological interactions is crucial to achieving the desired outcome of environmental introductions of transgenic organisms, including crop plants, pest control products, and beneficial microbial populations.

An example of past contributions of ecologists to environmental management can be drawn from early problems associated with pesticide use (Woodwell 1967, Woodwell et al. 1967). Ecologists responded to these problems by examining the transfer and concentration of pesticides in food chains and the evolution of pesticide resistance in target and nontarget organisms. Elucidating these processes resulted in the modern recognition of the value of biological control and the development of pest management programs based on the dynamics of pest populations, the introduction and preservation of beneficial organisms, and reductions in the amounts of the pesticides used (Huffaker 1980, National Research Council 1986*b*).

Ecological expertise will be important in future applications of biotechnology. The acquisition of effective gene libraries for use in biotechnology will be aided by recognizing and analyzing the products, functions, and evolutionary features of organisms in nature. For example, novel genetic defenses for crop plants may be obtained from the natural defenses of wild plants against insect pests. The microbial community also represents a largely untapped resource of useful genes. In addition, by investigating microbial competition and the necessary environmental conditions for population establishment, microbial ecologists will be able to assist in the successful establishment of populations of beneficial microorganisms. **The characterization and understanding of the ecological traits and requirements of transgenic organisms will often be critical to the development of successful, low-risk, genetically engineered organisms.**

Potential ecological effects

Most engineered organisms will probably pose minimal ecological risk. Many genetically engineered organisms will be modified domesticated species living under controlled agricultural conditions. Although domesticated animals sometimes establish feral populations, it would be difficult to convert most crop plants into organisms that can survive and reproduce without human support. However, in cases where an organism may persist without human intervention, or where there may be genetic exchange between a transformed organism and unaltered organisms, an assessment of environmental risk is required.

The following examples provide an overview of the types of undesirable outcomes to be avoided:

1) The creation of new pests. An example might be the creation of a salt-tolerant transgenic rice capable of escaping cultivated fields and invading estuaries.

2) Enhancement of the effects of existing pests through hybridization with related transgenic crop plants. One hypothetical class of examples would include the acquisition by weeds of engineered disease or herbicide resistance. Traits that enhance the competitiveness or physiological tolerances of weeds, such as salt, drought, or insect tolerance, will warrant consideration (Windle and Franz 1979).

3) Harm to nontarget species. For example, viruses with broadened host range could infect beneficial insects as well as the targeted pest.

4) Disruptive effects on biotic communities. The elimination of wild or desirable naturalized species through competition or interference is a possible outcome in some cases. For example, the introduction of the highly competitive nitrogen-fixing bacterium, *Bradyrhizobium* serogroup 123, into agricultural fields has made it difficult to introduce more effective rhizobia

(Ellis et al. 1984, Moawad et al. 1984). This negative economic outcome illustrates the importance of ecological information to sound economic analysis. Fish engineered to be larger or more cold tolerant also could have disruptive effects by displacing other fish species (Moyle 1986). The introduction of genes coding for an insecticidal toxin into forest or semi-domesticated tree crops (McGranahan et al. 1988) has the potential to alter plant community composition by diminishing seed predation, if the toxin is expressed in the seeds of the transgenic trees or in the seeds of wild relatives that have acquired the toxin genes by hybridization (Simmonds 1976).

5) Adverse effects on ecosystem processes. For example, the increased expression of microbial ligninase or constitutive denitrification could alter nutrient cycling adversely. Plants introduced for beneficial purposes may have adverse effects on ecosystem function, such as the disruptive effects on nitrogen fixation by the introduction of *Myrica* in Hawaii (Vitousek 1986). The availability of limiting nutrients may also be altered.

6) Incomplete degradation of hazardous chemicals leading to the production of even more toxic by-products. For example, the microbial degradation of trichloroethylene (TCE) and tetrachloroethylene (PCE) produces the more toxic vinyl chloride (Vogel and McCarty 1985).

7) Squandering of valuable biological resources. For example, the genes for toxins produced by strains of the bacterium *Bacillus thuringiensis* (*Bt*) have now been inserted into several crop plants and trees, conferring resistance against some insect herbivores. However, the genetically engineered crops as well as the unaltered bacteria could be rendered ineffective by creating conditions that accelerate the evolution of pest resistance (Gould 1988a, b, Colwell, in press).

Transgenic organisms can be designed to minimize the chance of environmental perturbations. The choice of the trait and parent organism used, the form of the genetic alteration, and the control of its expression all affect the likelihood that the genetically engineered organism will have undesirable effects. In addition, the conditions of the organism's introduction can be planned to minimize potential problems. Thus, **we believe that with careful design of transgenic organisms and proper planning and regulatory oversight of environmental releases, the introduction of many transgenic organisms can be carried out with minimal ecological risk.**

Scientific Issues

The complexity that arises in evaluating the efficacy and possible risks of environmental uses of biotechnology stems from the diversity of organisms to be

modified, the desired functions to be introduced, and the physical and biotic components of target environments. The organisms that may be engineered span the entire range of organisms from viruses, bacteria, and simple eukaryotes to multicellular plants and animals, including domesticated and wild species. The expertise necessary for evaluating the benefits and risks of biotechnology involves many scientific disciplines, including molecular biology, genetics, cell biology, evolutionary biology, physiology, population and community ecology, and ecosystem science (Hodgson and Sugden 1988).

The pending release of genetically altered organisms into the environment raises an array of potentially important scientific issues. The survival and reproduction of transgenic organisms, their interactions with other organisms, their potential for spread beyond the point of their introduction, and their effects on the physical environment will all individually and collectively influence whether the released organisms pose any risk to the environment. Knowledge of the genetics, physiology, and ecology of both the parent organism and the gene donor, and the characteristics of the environment that will receive the modified organism will be necessary for an evaluation of the potential effects of a transgenic form (National Institutes of Health 1985, National Academy of Sciences 1987).

Importance of phenotype

Some transgenic organisms will differ little from the parent organism, perhaps by only one gene. Others will differ much more. The important consideration is the character of the phenotypic changes expressed after manipulation of the organism's genotype, not simply how many genes have been added or deleted. Changing a market-oriented attribute of an agricultural plant, such as the amino acid content (for example, in corn), may have few important ecological consequences. On the other hand, phenotypic changes that result in increases in physiological tolerance, substrate utilization, or range extensions may have far-reaching effects on the phenotype's abundance, range, and interactions with other organisms and with the physical environment.

We contend that transgenic organisms should be evaluated and regulated according to their biological properties (phenotypes), rather than according to the genetic techniques used to produce them. Both the intended product-oriented phenotypic changes and other, possibly unintended, changes in the phenotype need to be considered. The new phenotype of the transgenic organism, together with the characteristics of the environment into which it will be introduced, should be the basis for evaluating the potential ecological effects of a proposed introduction. Nonetheless, because many novel combinations of properties can be achieved only

by molecular and cellular techniques, products of these techniques may often be subjected to greater scrutiny than the products of traditional techniques.

Characterization of the genetic change

Traditional methods of genetic alteration often produce multiple, unknown effects on the genotype and unexpected effects on the phenotype. In contrast, the precision with which genetic alterations can be made with molecular techniques is often cited as an argument for the safety of genetically engineered organisms (Brill 1985, Davis 1987). Pleiotropic effects (secondary phenotypic effects of a single genetic alteration), however, may easily be overlooked in focusing on intended primary effects, and some effects may be expressed only in particular environments (e.g., Stotzky and Babich 1985), even if the genotype is fully characterized.

Although the capability to produce precise genetic alterations increases confidence that unintended changes in the genome have not occurred, precise genetic characterization does not ensure that all ecologically important aspects of the phenotype can be predicted for the environments into which an organism will be released.

Fitness of genetically engineered organisms

The potential for a transgenic organism to survive and reproduce in the environment for which it is intended must be examined when considering the possible long-term consequences of an introduction. It has often been assumed that the addition of extra genes reduces the competitiveness of an organism due to the added cost of synthesizing additional nucleic acids and proteins (e.g., DaSilva and Bailey 1986, Davis 1987, Lenski and Nguyen 1988). Normal physiological processes may also be disrupted as a result of the expression of novel traits. For example, Bassford et al. (1979) observed that the growth of bacterial cells synthesizing a malE-lacZ hybrid protein was severely impaired, apparently because the hybrid protein became embedded in the cytoplasmic membrane and thus blocked sites needed for transport of essential components of the outer membrane. Similarly, Moyed et al. (1983) found that high-level expression of a tetracycline resistance gene could severely inhibit bacterial growth. They demonstrated that the level of phenotypic resistance to tetracycline actually decreases at very high levels of expression of the resistance gene. This example illustrates the potential complexity of the relationship between genotype and phenotypic expression.

In contrast, there are also important exceptions to the general observation that additional genes decrease competitiveness. Devanas and Stotzky (1986) found that nonessential eukaryotic DNA inserted into plasmid DNA had little effect on bacterial survival in lab-

oratory soil or saline environments. In the first field test of an engineered soil bacterium (*Pseudomonas fluorescens* with inserted lacZY gene), the population sizes of the introduced parent and transgenic organisms were identical for 30 wk (Kline et al. 1988). Furthermore, competitiveness in bacteria has even been enhanced with the possession of foreign DNA (Hartl et al. 1983, Edlin et al. 1984, Bouma and Lenski 1988, Marshall et al. 1988). For example, Hartl and colleagues (1983) found that the transposon Tn5 and its associated IS50 insertion sequences actually increase the growth rate of certain bacterial strains. The precise genetic and physiological bases of these exceptions are not known, but they raise the possibility that similar fitness-enhancing functions may inadvertently be transmitted along with an intended genetic alteration during molecular engineering.

Even in cases where fitness is reduced, the transgenic organism may persist in the environment. For example, if there is a 1% reduction in growth rate per generation of the transgenic organism relative to the wild type, hundreds or even thousands of generations may be required for the population of the introduced organism to be completely displaced. This phenomenon may be especially problematic when the turnover rate of populations is very slow, as is likely for many higher organisms and microbial species growing at very low resource concentrations.

In summary, many engineered organisms will probably be less fit than the parent organism, although some important exceptions may arise. Even if an engineered trait reduces an organism's fitness only slightly, many generations may pass before the introduced organism disappears completely due to decreased fitness.

Variation and selection

If novel genes can enhance competitiveness, why have organisms not evolved these functions already? Current ecological and evolutionary thought does not embrace the notion that organisms are perfectly adapted to their environment (Gould and Lewontin 1979, Harper 1982). There are important constraints on evolutionary processes that limit the ability of organisms to become perfectly adapted. In particular, adaptation requires the existence of appropriate genetic variants on which selection can act. Much of the power of modern molecular biology lies in its ability to circumvent this constraint and produce radically new genetic variants.

Natural selection will act on transgenic organisms as it does on all others. **Selection after the release of the transgenic organism will tend to increase fitness, not decrease it, by reducing the costs associated with the novel traits.** The reduction in costs could result from a mutation that reduces the level of expression of a costly gene product, or one that compensates for some critical physiological process disrupted by the engineered gene product. Such changes in the burdens associated with novel phenotypes have been documented in both microbes and higher organisms (Lenski and Nguyen 1988). Mutants of *E. coli* that are resistant to the virus T4 are less competitive than the virus-sensitive parental strain when the two strains are allowed to compete in the absence of the virus (Lenski and Levin 1985). However, this cost of resistance has been overcome, at least in part, by a subsequent adaptation enhancing competitiveness without reducing resistance (Lenski 1988). Similarly, McKenzie et al. (1982) have documented that a reduction in competitiveness in the sheep blowfly (*Lucilia cuprina*) associated with resistance to the insecticide diazinon, was eliminated by subsequent adaptation of the blowfly. If increases in fitness do occur in transgenic organisms introduced into the environment, they will probably increase population growth rate and biological competitiveness, or produce other ecological effects that should be considered in assessing risks.

Organisms bearing phenotypes new to a biological community may also act as agents of natural selection. Gould (1988a, b) modeled the evolution of resistance in insects to *Bacillus thuringiensis* (*Bt*) toxin following the introduction of agricultural cultivars carrying the gene for its production and found that the greater exposure to *Bt* toxin presents herbivorous insects with a powerful new selective pressure that may result in the acquisition of *Bt* resistance. Such resistance could reduce the efficacy of engineering the *Bt* toxin into agricultural cultivars and could also change the role that naturally occurring *Bt* toxin plays in the ecosystem.

Gene stability and transfer

Assessing the risks associated with the introduction of transgenic organisms is made more complex by the capacity of many modified organisms to exchange genes with unaltered organisms. For example, crop plants vary enormously in their potential for hybridization with wild relatives (Harlan 1965). At one extreme is the crop turmeric (a component of curries) which is maintained in cultivation entirely through vegetative propagation; at the other extreme is alfalfa, which is an obligate outbreeder (Harlan 1975). Other species such as peas, lentils, and wheat are considered inbreeders but are capable of some outcrossing. Despite the intensive search by plant breeders for the wild relatives of crops, knowledge of the distribution and the ecology of these wild species is surprisingly incomplete. We do know, however, that few major crops originated in temperate North America (Hodge and Erlanson 1956). Many crop species, exemplified by rice, tomato, and rubber, arose in tropical or subtropical areas where they

often are cultivated in fields adjacent to their wild relatives (Harlan 1975). Care should be taken to prevent crops from passing inserted genes to their wild relatives via hybridization. Such transfer becomes important because genes that confer a new ability, such as insect or disease resistance, or salt or drought tolerance, could also change the physiological tolerances or geographic distributions of wild plants, causing them to become economically important weeds or altering their roles in natural communities (Hauptli et al. 1985, Center for Science Information 1987, Ellstrand 1988).

Transgenic bacteria may exchange genetic material with naturally occurring strains by conjugation, transduction, or transformation (Lenski 1987, Miller 1988). If lateral transfer from the modified organism to other organisms occurs, an engineered gene may persist in the natural environment even after the genetically engineered organism itself is no longer present. This is one category of extenuating circumstances that will need to be considered in risk assessment.

Current knowledge of gene exchange by microbes comes largely from laboratory studies with a few vectors heavily used in molecular genetics. Little information exists on the prevalence of these vectors outside the laboratory. We know, however, that microbial taxa are variable in the extent to which they exchange chromosomal and extra-chromosomal genes (Lenski 1987), and that biological, physical, and chemical properties of the environment can all influence the rate of gene exchange (Stotzky and Krasovsky 1981). **The available scientific evidence indicates that lateral transfer among microorganisms in nature is neither so rare that we can ignore its occurrence, nor so common that we can assume that barriers crossed by modern biotechnology are comparable to those constantly crossed in nature.** Techniques are being developed to prevent or reduce the potential for gene transfer (Bej et al. 1988, Office of Technology Assessment 1988). By engineering a stable construct, verifying its stability in the presence of known vectors, controlling the densities of introduced organisms, and characterizing potential environments that will receive the transgenic organisms, field experiments with low risk for gene exchange are possible.

Effects of the scale and frequency of introductions on establishment

In some cases, the establishment of persistent populations of genetically engineered organisms will be the goal of an environmental introduction. For example, transgenic organisms intended for the biological control of particular pests may be designed to persist at low population levels in the absence of a pest outbreak. In other cases, engineered organisms will be designed to carry out a particular mission, then die out or recede to minimal population levels. Examples include the breakdown of toxic waste by microbes (e.g., Omenn 1988), or the production of commercial products by annual plants (see Center for Science Information 1987). Whether establishment is intended or not, the ability of a genetically engineered organism to become established in natural or managed ecosystems becomes a critical issue.

Case histories of both disease epidemics and invasions of higher organisms suggest that the scale of the introduction can determine whether the introduction yields a self-sustaining population (e.g., Crowell 1973, Schoener and Spiller 1987). The minimum effective inoculum threshold varies widely among organisms both with regard to the density of the inoculum and the geographic range over which it is introduced (Dunigan et al. 1984, Simberloff 1986). Threshold numbers vary for different reasons. In some cases, rapid predation or another source of high mortality can be overcome only by a large founder population. In other situations, a large founder population will provide sufficient genetic variation from which genotypes can be selected that can tolerate the new environment (Salisbury 1961, Baker 1986).

The frequency of introductions also affects the ability of an organism to establish a population, because frequent releases increase the likelihood that a threshold number of individuals will find sites favorable for establishment and reproduction. Moreover, the season and environmental conditions at the time of introduction could influence whether the population will become established (Ridley 1930, Crawley 1988). The method of introduction also influences establishment: microorganisms injected into the xylem of plants, released as aerosols, or mixed with soils will experience very different initial conditions, resulting in different probabilities of establishment.

Under most circumstances, small-scale field tests will involve the introduction of a limited number of organisms into a limited environment, on one or a few occasions. These conditions will reduce the probability that a transgenic population will become established from a small-scale field test. **Thus we encourage the use of small-scale field tests, when justified by previous laboratory and/or greenhouse studies, under conditions that minimize dispersal and under appropriate regulatory oversight.** Field experimentation and data from population monitoring are also crucial in evaluating the potential for larger scale environmental effects.

Fate of introduced transgenic organisms: potential for delayed effects

If a transgenic organism can be eliminated following an experimental introduction, the risk associated with its field testing will usually be insignificant. Elimination may be practical for larger plants and animals. Insects,

microorganisms, and viruses, however, may be difficult to exterminate following their introduction. Difficulty or uncertainty about the extermination of an introduced organism should result in closer scrutiny of the proposed introduction.

The ability of organisms to persist in nature is often surprising, and we expect that cases of persistence of transgenic organisms will emerge. In a number of documented cases, introduced microorganisms were thought to have disappeared because they could not be detected by culture methods, but much later the progeny of these organisms or their gene sequences appeared under the appropriate conditions for growth (Devanas et al. 1986, Chatterjee 1988). In addition, there are a number of disease-causing organisms that are no longer detectable, yet the disease occurs (R. R. Colwell et al. 1985). Even among higher organisms, a small number of individuals, below the limits of detection, may persist for a period of time, then suddenly increase when appropriate environmental conditions occur (Mack 1985, Moody and Mack 1988). **Rather than focus upon whether an introduced transgenic organism is likely to disappear completely, emphasis should be placed on whether its population is likely to remain viable or increase in size under appropriate environmental conditions.**

Furthermore, many types of ecological effects may be indirect, taking some time to appear. Examples include bioaccumulation, species replacement, and perturbations of geochemical cycles that have been observed only after many years of chronic pollution or, in some cases, years after a single introduction of a new species into the environment. Unlike the effects of releasing chemicals into the environment, the direct effects of self-replicating introduced organisms may not necessarily decrease with time or with distance from the point of introduction. **The absence of an immediate negative effect does not ensure that no effect will ever occur.**

Community and ecosystem level effects

Establishment of persistent populations depends upon interactions between the engineered organism and other species in the biological community, and upon the integration of the organism into the receiving ecosystem. In turn, successful establishment will sometimes have significant effects on species interactions and ecosystem function (Williamson 1988). Although the focus of past discussions has often been on the capability of engineered organisms to compete successfully with naturally occurring organisms and on their potential for pathogenicity (e.g., Davis 1987), other classes of species interaction are also important. Mutualistic interactions, in particular, underlie many critical ecosystem functions, including nitrogen fixation,

inorganic nutrient uptake through mycorrhizae, pollination, cellulose decomposition, and digestive processes in both invertebrate and vertebrate animals (Futuyma and Slatkin 1983). **Engineered organisms that alter mutualistic associations, either by design or unintentionally, require careful evaluation.**

Initial information on the potential for establishment and possible effects of an engineered organism should be obtained from laboratory microcosm and mesocosm studies, or from tests in a contained greenhouse. Assuming these preliminary studies reveal no unacceptable risks, further information should be obtained from carefully planned small-scale field trials that include evaluation of both intended and unintended effects on other species in the ecosystem.

The widespread establishment or use of transgenic organisms might result in effects on ecosystem function. For example, if an improved *Bradyrhizobium* strain were to dramatically increase nitrogen fixation and enhance legume productivity, it is likely that soil nitrogen would be enriched because nitrogen mineralization would probably continue at its usual rate. Excessive soil nitrogen might then lead to establishment of new weeds, increased leaching of nitrate, and increased flux of nitrogen oxides into the atmosphere.

In microbial communities, we often understand the functional roles that microorganisms play better than we know the species composition of the community. Concern has frequently been expressed regarding the potential for introductions of transgenic organisms to displace resident species in the receiving community, particularly microbial species performing key functional roles such as nitrogen fixation, lignin decomposition, or pesticide degradation (Levin and Harwell 1986). Natural communities have considerable "redundancy" in that many species perform similarly in key roles. Such "functionally equivalent" species, however, undoubtedly differ ecologically in other respects. Differences in functional roles will be important in some cases, especially among higher organisms, but may be of little importance in others. For example, the value of a species at risk for displacement may be economic or aesthetic, in addition to any role it may play in community and ecosystem function. **Because redundancy of function appears to be common in microbial communities, in many cases there would be little concern over microbial species displacement caused by an introduced transgenic organism.**

The merits and limits of existing models

Agricultural breeding.—Numerous models have been used in evaluating the potential effects of the introduction of transgenic species into the environment (Goldburg 1988, Regal 1988, Colwell, *in press*). Most of these have some merit, but all have limits in their

applicability. One general model is based on the long-term experience derived from traditional breeding (e.g., Ellingboe 1985, National Academy of Sciences 1987). This vast and often anecdotal record provides useful information on the effects of moving domesticated species from one location to another, and on moving genes via hybridization to create new strains. This record provides useful information for the evaluation of genetic alterations similar to those that might have been produced by traditional means, and such alterations are likely to pose few ecological problems. The agricultural model, however, reflects techniques that lack the wide-ranging ability of molecular biology to transfer traits among very different species. Consequently, an overall record of little or no hazard stemming from the release of the products of traditional agricultural breeding does not legitimately warrant exemption from oversight for future introductions of transgenic organisms that these traditional techniques could not have produced.

Introduced species model.—The introduced species model provides another possible analog for the potential ecological effects of the environmental release of genetically engineered organisms (Sharples 1983, 1987, R. K. Colwell et al. 1985, Regal 1986). Most terrestrial and freshwater biological communities include self-propagating species from two general sources. Some are native species that were present before human intervention. Others are naturalized, that is, they were introduced either deliberately or accidentally by humans and have become integrated into the biological community. *Bradyrhizobium japonicum* (a symbiotic nitrogen-fixing bacterium of soybean), certain crops that have escaped from cultivation including Jerusalem artichoke and blackberries, many agricultural weeds, and a few animals including pheasants and brown trout, fit this category. Occasionally, these introduced species have become pests.

A functional analogy may be drawn between the reasons for the naturalization of non-native organisms and the possibility that introduced transgenic organisms might become naturalized. For example, a plant may persist in a new range because in the course of immigration it has escaped its native herbivores and has not attracted new herbivores. A transgenic plant may experience the same outcome with insertion of genes that control the production of proteinase inhibitors (Thornburg et al. 1987). Similar functional analogies could be drawn between the escape from fungal parasites or the extension of growing season, and the impending or contemplated insertion of genes imparting fungal resistance or frost tolerance. In drawing these functional analogies, we are not maintaining that all transgenic plants will become naturalized; we contend instead that our ability to assess this risk (of whatever size) will be

enhanced by realizing that under some circumstances imparting a new trait to a plant may confer the same advantages that some plants have experienced through their dispersal to areas far outside their usual ranges (Simberloff 1985, Williamson 1988). This analogy can also be applied to other groups of higher organisms.

An organism engineered to prosper in a new habitat, geographic area, or season is effectively an introduced organism in that it probably will enter into new biotic and abiotic interactions. Both native species and species introduced from distant habitats may become pests (Pimentel 1986). Regulatory and risk assessment structures that rely on the distinction between "native" and "non-native" must therefore be used with caution. **Historical experience with the introduction of non-native species may be relevant for modified native or naturalized organisms, as well as for the introduction of genuine non-natives.**

Laboratory experience.—A commonly cited argument for the safety of genetically engineered organisms stems from the laboratory experience gained since the mid-1970s with recombinant-DNA microorganisms. Although some of these organisms have probably escaped containment, negative effects have not been detected (Davis 1987). These escapes have probably ended in local extinction because the escapees arrived in incompatible habitats in numbers below the threshold densities for establishment, and because these organisms were often intentionally designed to have lower fitness than their genetically unaltered counterparts.

Although obtaining laboratory data is an important first step in evaluating the ecological traits of genetically engineered organisms, these data alone cannot accurately predict the fate of an introduced organism released in nature. The complexity and dynamic character of the physical, chemical, and biological environment has not been duplicated in the laboratory. Laboratory soil microcosms can provide reasonably natural habitats to study the ecological properties of a soil microorganism. Extensive experience in plant pathogen research, however, has shown that the laboratory is not a reliable environment in which to study the ecology of a leaf pathogen. Thus the usefulness of data from laboratory studies for predicting environmental fate will vary widely.

REGULATORY POLICY

Previous sections discussed scientific principles derived from the field of ecology that should be used in planning for the development, testing, and risk assessment of the introduction of genetically engineered organisms into the environment. Ecological oversight of such introductions should be directed at promoting effectiveness while guarding against potential problems. The diversity of organisms that will be modified,

functions that will be engineered, and environments that will receive modified organisms makes ecological risk evaluation complex. Clearly, however, different organisms, traits, and environments present different probabilities of adverse effects. This complexity has made it difficult to establish categories for different degrees of regulatory oversight. Ecological knowledge, however, does allow us to endorse certain principles that should be useful in developing regulatory policy and in recognizing the degree of risk associated with different attributes of engineered traits, organisms, and environments.

Scaling of regulatory oversight—a recommendation

Regulatory agencies are currently working to develop risk assessment guidelines and policies within the framework of a diverse set of legal mandates and historical precedents (National Institutes of Health 1985, 1986, Office of Science and Technology Policy 1986, McGarity 1987, Ager 1988, Kingsbury 1988, Office of Technology Assessment 1988). While we cannot now recommend the complete exemption of specific organisms or traits from regulatory oversight, we support and will continue to assist in the development of methods for scaling the level of oversight appropriate to individual cases according to objective, scientific criteria. The goal is to ensure safety while minimizing unnecessary or counterproductive regulatory burdens. In this section we attempt to outline a set of genetic, phenotypic, and environmental criteria that might provide a scientific basis for uniform policy. Table 1 presents a preliminary attempt at developing a comprehensive overview of attributes that may permit a priori scaling of regulatory oversight for the testing and use of genetically engineered organisms in the environment. (The design of this table was inspired by a similar table prepared by the Recombinant DNA Monitoring Committee [Australia] 1987.)

As we have stressed, ecological risk assessment and regulatory oversight of genetically engineered organisms, for either field testing or commercial use, require consideration of many factors. Some may be quantified, such as numbers of organisms to be released, size of test plots, or susceptibility of organisms to biocides should mitigation be necessary. Many factors are, however, necessarily qualitative or only roughly quantifiable, such as the level of domestication of the parent species, or the infectivity or virulence of the parent organism. Nonetheless, we consider it essential to develop some practical means of scaling the level of regulatory oversight according to the probability of adverse effects. Such scaling is equally essential as a means of focusing risk assessment on only those features of organisms and environments that require it.

Three features of Table 1 must be stressed. First, the scaling of each attribute is only qualitative or semi-quantitative (ordinal). Second, the scales for any two attributes (any two lines in the table) are not necessarily commensurate, either in terms of the probability of adverse consequences or the severity of such an outcome, if realized. Third, the table is multidimensional. Overall estimates of risk for a particular genetically engineered organism in a particular environmental context require, in effect, a simultaneous mapping of the attributes of the case on all the individual scales of the table. The estimate of risk based on one scale may be greatly modified by the scores on other scales. For example, if the parent organism is a pathogen ("habit" scale in Part B), then a broadened host range ("host range" scale in Part C) will yield a higher estimate of risk than would a non-pathogenic organism with a comparably broadened host range.

We must warn against combining scores on the different scales of the table. Not only are they incommensurate, but scores on different scales are also neither strictly multiplicative (as fully independent probabilities would be), nor strictly additive. Experience with real cases will ultimately provide information on actual levels of risk for many regions of this multidimensional space.

In the meantime, we urge that any case that falls at the right-hand end of *one or more* scales in Table 1 should receive appropriate regulatory scrutiny in regard to the attribute(s) in question. Ecological safety, as well as public confidence in a fledgling industry, will be fostered by this approach. Clearly many in the biotechnology industry see the issue in much the same way that we do. In the words of Robert Goodman and his colleagues (Goodman et al. 1987) at Calgene, Inc.:

> *The unusual power of the technology, uncertainty over the behavior to be expected from organisms modified in novel ways, and the past 40 years of experience with chemicals in the environment make it reasonable and indeed desirable that genetically modified organisms be introduced cautiously.*

Comments on current regulatory policy

The current regulatory framework uses biological characteristics and existing institutions (e.g., federal agencies, universities, industrial laboratories) to structure the oversight process (Office of Science and Technology Policy 1986). Certain aspects of the regulatory framework warrant comment from an ecological perspective.

Case by case review.—The current regulatory practice for review of proposed environmental introductions uses a case by case analysis of the organism, environment, and experimental protocol. **Case by case review is currently the most scientifically sound regu-**

TABLE 1. Attributes of organisms and environments for possible consideration in risk evaluation.*

A. Attributes of genetic alteration

Level of possible scientific consideration

Attribute	Less		More
Characterization	Fully characterized		Poorly characterized or unknown
Genetic stability of alteration	High (e.g., chromosomal)		Low (e.g., extra-chromosomal)
Nature of alteration	Gene deletions (unless host range altered)	Single gene added	Multiple genes added
Function	None (no expression or regulation)	Regulation of existing gene product	Synthesis of gene product new to parent organism
Source of insertion	Same species	Closely related species	Unrelated species
Vector	None	Non-self-transmissible	Self-transmissible
Source of vector	Same species; non-pathogen	Closely related species; non-pathogen	Unrelated species or pathogen
Vector DNA/RNA in altered genome	Absent	Present, but non-functional	Functional

B. Attributes of parent (wild type) organism

Level of possible scientific consideration

Attribute	Less		More
Level of domestication	Unable to reproduce without human aid	Semi-domesticated; wild or feral populations known	Self-propagating, wild
Ease of subsequent control	Control agents known		No known control agents
Origin		Indigenous	Exotic
Habit	Free-living		Pathogenic, parasitic, or symbiotic
Pest status	Relatives not pests	Relatives pests	Pest itself
Survival under adverse conditions	Short term		Long term (e.g., spores, cysts, seeds, dormancy)

TABLE 1. Continued.

Geographic range, range of habitats	Narrow		Broad or unknown
Prevalence of gene exchange in natural populations	None		Frequent

C. Phenotypic attributes of engineered organism in comparison with parent organism

	Level of possible scientific consideration		
	Less		More
Fitness	Reduced irreversibly	Reduced reversibly	Increased
Infectivity, virulence, pathogenicity, or toxicity		Reduced irreversibly	Reduced reversibly	Increased
Host range	Unchanged		Shifted or broadened
Substrate, resource	Unchanged	Altered	Expanded
Environmental limits to growth or reproduction (habitat, microhabitat)	Narrowed but not shifted		Broadened or shifted
Resistance to disease, parasitism, herbivory, or predation	Decreased	Unchanged	Increased
Susceptibility to control by antibiotics or biocides, by absence of substrate, or by mechanical means	Increased	Unchanged	Decreased
Expression of trait	Independent of environmental context		Dependent on environmental context
Similarity to phenotypes previously used safely	Identical	Similar	Dissimilar

D. Attributes of the environment

	Level of possible scientific consideration		
	Less		More
Selection pressure for the engineered trait	Absent		Present

TABLE 1. Continued.

Wild, weedy, or feral relatives within dispersal capability of organism or its genes	Absent		Present
Vectors or agents of dissemination or dispersal (mites, insects, rodents, birds, humans, machines, wind, water, etc.)	Absent or controllable		Present, uncontrollable
Direct involvement in basic ecosystem processes (e.g., nutrient cycling)	Not involved	Marginally involved	Key species
Alternative hosts (partners), if organism is involved in symbiosis (mutualism)	Absent		Present
Range of environments for testing or use; potential geographical range	Very restricted		Broad, widespread
Simulation of test conditions	Not difficult to simulate realistically		Very difficult to simulate realistically
Public access to test site	Tightly controlled	Limited	Uncontrolled
Effectiveness of monitoring and mitigation plans	Proven effective		Untested or unlikely to be effective

* Position on scale is only qualitative or semi-quantitative. The importance of position on one scale may be contingent on another scale. The importance of particular scales will vary with different cases.

latory approach because of the diversity of products that can be developed and the complexity of predicting their ecological fate. We expect that workable guidelines, to include categories of organisms requiring minimal screening and review, as well as those requiring more intensive review, will be developed after experience is gained from field experiments and from research that is stimulated by ecological risk assessment. These guidelines should be based upon the types of attributes of transgenic organisms and environments described in Table 1. The regulatory system should

remain flexible so that it can easily accommodate new information.

Product vs. process. —Current regulatory policy is focused on organisms produced by recombinant DNA technology (Office of Science and Technology Policy 1986). For the reasons discussed in the Scientific Issues section, **the phenotype of the transgenic organism, not the technique used to produce it, is the appropriate focus of ecological risk assessment and regulatory oversight.** Nonetheless, because many novel combinations of properties can be achieved only by molecular and cel-

lular techniques, products of these techniques may often be subjected to greater scrutiny than the products of traditional techniques.

Coding vs. non-coding regions. — Under current government policy, well-characterized, non-coding regulatory regions of the genome, regardless of their source, are assumed to pose no higher risk than does the unaltered recipient organism (Office of Science and Technology Policy 1986). Regulatory regions of the genome, however, serve to control the level and timing of the production of gene products, in some cases turning production on or off entirely. Thus, ecologically important aspects of the phenotype, such as substrate utilization, may be altered when a non-coding regulatory region is inserted (Colwell et al. 1987). In fact, an important and increasingly dominant theory of the origin of evolutionary novelties and higher taxa is that changes in regulatory regions are more important than changes in regions that code for gene products in producing these major evolutionary shifts (King and Wilson 1975, Paigen 1986). In addition, random insertion of promoters into the chromosome can cause expression of more than the inserted construct, including previously silent genes. Thus, **non-coding regulatory regions should not be exempted from ecological risk analysis and regulatory oversight.**

Categorization by lists of taxa and vectors. — Taxonomic lists of organisms and vector type are used by some regulatory agencies in determining the level of review required for a proposed introduction (e.g., National Institutes of Health 1986). Such categorization of safety by taxonomic listing is sound in some cases, but not in others. The biology of most crop plants is well understood, whereas the ecological characteristics of many bacteria are not, and the ecological characterization of vectors is nonexistent. A belief that any given gene exchange demonstrable in the laboratory is commonplace in nature is an hypothesis that has yet to be confirmed or rejected, and thus should not be the cornerstone of regulatory policy. **Lists of taxa and vectors categorized for environmental safety are only appropriate when substantiated by an ecological data base for that organism, including data on the attributes listed in Table 1.**

Commercial vs. noncommercial research. — The current regulatory framework partitions regulatory oversight according to funding source and existing statutory authority (Office of Science and Technology Policy 1986). One particularly troublesome aspect of this practice is the separation of commercial (privately funded) research from noncommercial research. From a scientific point of view, the risk from the introduction of a particular transgenic organism is the same whether the introduction is made by a scientist from the private

sector or a university professor. Scientific concerns should guide oversight of planned introductions into the environment regardless of funding source. **We urge that ecological risk assessment be sound and equal for both commercial and noncommercial research.**

Development of regulatory oversight

The first deliberate environmental introductions of genetically engineered organisms have been made using organisms altered in ways that present minimal ecological risk (e.g., Kluepfel et al. 1988, Lindow et al. 1988). Maintaining this approach for now allows time for the development of appropriate ecological risk assessment methods for future use, possibly including a set of categories and risk factors such as we have outlined in Table 1. We are concerned, however, that because the first introductions are especially likely to be innocuous, there may be a tendency to overgeneralize from these examples. **The absence of problems at an early stage suggests that the screening mechanisms are working correctly, but should not be interpreted to mean that the introduction of all genetically engineered organisms is inherently safe.** The appropriate use of early experience from field introductions is to establish principles and refine the regulatory structure in order to balance true risk with the extent of review.

We encourage the gradual development of biotechnology products and of regulatory oversight. As the biotechnology industry develops, continuing regulatory oversight, as well as long-term research and monitoring, are necessary for responsible risk management.

Consistency of regulation over political boundaries

Ecological effects and the geographic ranges of organisms transcend political boundaries; therefore, we consider it essential to promote and achieve international coordination of risk assessment and regulation of biotechnology. Because the potential hazards of engineered organisms are often environment-dependent, and ecosystems and biotas vary geographically and climatically, an organism that is safe in one country (or one state) is not necessarily safe in another. Thus both the commercial import and export and the inadvertent dissemination of engineered organisms or their genes across political boundaries present special concerns that require cooperation and coordination. Special consideration must be given to the protection of rare genetic resources, such as the wild ancestors of domesticated species, and threatened gene pools of other wild species. **We urge local, state, national and international cooperation in regulation, risk assessment, and risk management of the ecological effects of the introduction of genetically engineered organisms.**

Interdisciplinary research and education

There is a pressing need for interdisciplinary collaboration between molecular biologists, cell biologists, physiologists, ecologists, evolutionary biologists, and systematists in the development and environmental introduction of genetically engineered organisms. The new intellectual challenges and technical capabilities arising from this prospect suggest exciting research possibilities in key areas of ecology and evolutionary biology. Examples of such research include the study of population structure, community structure, and genetic diversity; gene flow, selection, speciation, hybridization, and other evolutionary processes; microbial ecology and evolution; the effects of limiting factors on abundance and distribution; the susceptibility of communities to invasion and changes in community structure; and the biological mechanisms of ecosystem processes. In turn, as we have stressed throughout this report, molecular and cell biologists stand to profit from the perspective and expertise of ecologists and evolutionary biologists in attaining both effectiveness and safety in the development of new products of biotechnology.

Interdisciplinary discourse is never easy to achieve and maintain. Cross-disciplinary conferences, graduate training programs, and one-on-one collaboration will be needed. The Ecological Society of America is ready to work with other scientific organizations and with regulatory agencies in promoting interdisciplinary research, education, and the incorporation of scientific information into the policy-making process. **For society to realize the full benefits of biotechnology, interdisciplinary research and graduate training programs are needed to expand the expertise of the scientific community at large.**

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