

Gene Flow from Genetically Modified Rice and Its Environmental Consequences

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*Within the next few years, many types of transgenic rice (*Oryza sativa*) will be ready for commercialization, including varieties with higher yields, greater tolerance of biotic and abiotic stresses, resistance to herbicides, improved nutritional quality, and novel pharmaceutical proteins. Although rice is primarily self-pollinating, its transgenes are expected to disperse to nearby weedy and wild relatives through pollen-mediated gene flow. Sexually compatible *Oryza* species often co-occur with the crop, especially in tropical countries, but little is known about how quickly fitness-enhancing transgenes will accumulate in these populations and whether this process will have any unwanted environmental consequences. For example, weedy rice could become much more difficult to manage if it acquires herbicide resistance, produces more seeds, or occurs in a wider range of habitats because of the spread of certain transgenes. Rice-growing countries urgently need publicly available ecological assessments of the risks and benefits of transgenic rice before new varieties are released.*

Keywords: *Oryza sativa*, transgenic rice, wild rice, hybridization, fitness

Rice (*Oryza sativa* L.) is one of the world's most important crops, providing a staple food for nearly half of the global population (FAO 2004). In many developing countries, rice is the basis of food security and is intimately associated with local ways of life. During the "green revolution" of the 1960s and 1970s, new semidwarf and photoperiod-insensitive rice varieties provided large yield increases and corresponding decreases in food shortages in many regions of the world. This dramatic increase in productivity can be attributed to crop breeding, to the intensification of fertilizer and pesticide use, and to better irrigation (Conway 1997). Moreover, even higher yields have been obtained in some countries by using hybrid rice varieties. Rice is the first crop species for which the complete genome has been sequenced (IRGSP 2005), and further efforts to improve yields are expected to benefit from ongoing advances in genomics and DNA marker-assisted selection.

Conventional rice breeding relies on sexual crosses between related species for introducing new genetic variation into breeding lines. Despite great progress, this approach is unlikely to produce yields sufficient to meet the demands of growing global populations, especially in developing countries (Conway 1997, FAO 2004). Also, the long-term stability of high-yielding rice production is constrained by losses caused by weeds, insects, and diseases, all of which can fluctuate and evolve to resist attempts to eradicate them. To overcome the inherent limitations of conventional rice breeding, genetic engineering has been used to isolate desirable genes from other organisms and insert these genes into specific rice varieties. Useful genes from viruses, bacteria, unrelated crops, and other organisms (including humans) can be iden-

tified, altered for better expression, and inserted into the rice genome, and artificial genes also can be created (table 1). Thus, transgenic methods have enormous potential to contribute to rice breeding by broadening the available gene pool. This "gene revolution" offers great possibilities for crop breeding and global food security (Conway 1997, Brookes and Barfoot 2003, FAO 2004). Unlike conventional breeding, however, the intentional release of such genetically modified (GM), or transgenic, crops is closely regulated by government agencies and international treaties.

Experimental lines of transgenic rice have been developed with traits such as increased yield, high protein content, pharmaceutical proteins, high vitamin content, disease and insect resistance, herbicide resistance, and salt tolerance (table 1; Jia et al. 2004). In the United States, over 200 applications for experimental field tests of transgenic rice have been received since 1992 (table 1; ISB 2004), but transgenic rice has yet to be grown anywhere in the world on a commercial scale. As of this writing, the only type of transgenic rice to be deregulated anywhere in the world is a herbicide-resistant variety (commonly known as LibertyLink rice, deregulated in the United States) with resistance to glufosinate. Other types

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Table 1. Examples of transgenic traits in rice, based on applications for field trial experiments and selected publications.

Transgenic trait	Additional details	Gene donor	Reference
Herbicide resistance			
Glufosinate resistance	Commercial names: LibertyLink, Basta	Bacterium	ISB 2004
Glyphosate resistance	Commercial name: Roundup Ready	Bacterium, CBI	ISB 2004
Imidazolinone resistance	Nontransgenic variety also exists	Maize	ISB 2004
Sulfonylurea resistance		Human	Inui et al. 2001
CBI		CBI	ISB 2004
Pest resistance			
Fungal disease resistance	<i>Rhizoctonia solani</i> (sheath blight)	Rice, <i>Arabidopsis thaliana</i> , bean, tobacco, barley	ISB 2004
	<i>Pyricularia oryzae</i> (blast)	Unknown	ISB 2004
Bacterial disease resistance	<i>Xanthomonas oryzae</i> (bacterial blight)	Rice, alfalfa	ISB 2004
Viral disease resistance	Several types	Virus coat proteins	OECD 1999
Insect resistance	Lepidopteran	Bacterium, cowpea	Xu et al. 1996, ISB 2004
	Coleopteran	Bacterium, cowpea	Ghoshal et al. 2001, ISB 2004
	Homopteran (brown planthopper, a disease vector)	Snowdrop lectin (<i>Galanthus nivalis</i>)	Wu et al. 2002
Stress tolerance			
Salinity tolerance		CBI	ISB 2004
Drought tolerance		CBI	ISB 2004
Cold tolerance		Rice	Hoshida et al. 2000
Abiotic stress tolerance		Unknown	SAGPyA 2003
Yield increase			
Yield increased		CBI, maize	ISB 2004
Photosynthesis and yield enhanced by C4 enzymes		CBI, maize	Ku et al. 2001, ISB 2004
Other traits			
Altered storage protein		Pea, rice	ISB 2004
Pharmaceutical proteins		Human, CBI, <i>Forsythia intermedia</i>	ISB 2004
Altered carbohydrate metabolism		CBI	ISB 2004
Novel protein		Human, CBI	ISB 2004
Altered polyamine metabolism		CBI	ISB 2004
Increased starch level		CBI	ISB 2004
Altered morphology		CBI	ISB 2004
Male sterility	For hybrid seed production	CBI	ISB 2004
Value-added protein		Human	ISB 2004
Heavy metal bioremediation		Bacterium	ISB 2004
Altered seed composition		CBI	ISB 2004
Enhanced beta carotene		Daffodil (<i>Narcissus pseudonarcissus</i>)	Burkhardt et al. 1997
Enhanced iron and zinc		Soybean	Vasconcelos et al. 2003
Low allergen		Unknown	OECD 1999
Low protein	For sake brewing	Unknown	OECD 1999

CBI, confidential business information.

Note: This list is not comprehensive, and it does not include several selectable or visual markers. Traits for which ISB 2004 is the reference were listed in field trial applications to the USDA Animal and Plant Health Inspection Service.

of transgenic rice are currently undergoing premarket improvement and regulatory approval, including the famous “golden rice,” which is intended to alleviate vitamin A deficiencies in people in developing countries (Burkhardt et al. 1997). In the United States, a biopharmaceutical company, Ventria Bioscience, has applied to state agencies for permission to grow more than 100 acres of pharmaceutical-producing rice for commercial purposes in California (permission denied) and Missouri (application pending).

Many transgenic rice varieties are likely to be released for commercial production within the next 5 to 10 years, depending on the rates at which they are approved by regulatory agencies and then commercialized (Brookes and Barfoot 2003, Jia et al. 2004). For example, China has invested heavily in developing disease- and insect-resistant varieties (figure 1), and many of these appear to be close to approval by the Chinese government (Jia et al. 2004). Meanwhile, the use of GM crops has stimulated worldwide debate about biosafety



Figure 1. Cultivated rice with transgenic resistance to insects. The experimental green plants on the left have two transgenes for lepidopteran resistance, Bt and cowpea trypsin inhibitor, while the brown plants on the right are nontransgenic controls. The controls have been killed by leaffolder larvae. This photograph was taken at a small-scale field experiment in Fuzhou, Fujian Province, China. Photograph: Wang Feng.

assessments, intellectual property rights, international trade, and ethical issues (NRC 2002). Here we use the term “biosafety” to refer to the effects of GM crops and their products on human health and the environment. Biosafety issues and public perceptions have the potential to create a bottleneck for further development of transgenic biotechnology, and for wider uses of GM rice in particular.

In this article, we focus on possible environmental risks that are related to gene flow from transgenic rice. Like other crop genes, novel transgenes can spread via pollen and seed dispersal to populations of related crops, weeds, and wild relatives (Ellstrand 2003). Ecologists expect the environmental consequences of gene flow from most GM crops to be negligible or neutral (NRC 2002, Snow et al. 2005). In certain cases, though, the unintended spread of transgenes could be undesirable from the standpoint of biosafety. In fact, possible consequences of gene flow from certain GM crops are often cited as a major environmental concern (NRC 2002, Hancock 2003). Therefore, it is useful to address questions related to transgene escape and its environmental consequences while new types of transgenic crops are being developed. The general questions we raise and the approaches for answering them are common to many species of crops that hybridize with wild or weedy relatives, including sorghum, wheat, oats, rye, canola, sugar beet, sunflower, squash, strawberry, carrot, lettuce, radish, alfalfa, clover, creeping bentgrass, poplar, and pine (Ellstrand 2003).

The gene pool of cultivated rice and its wild relatives

A first step in the assessment of gene flow and its consequences is to determine which cultivated or wild species can hybridize with the crop. To answer this question, it is necessary to examine the gene pool of rice and its related species. Asian cultivated rice, *O. sativa*, originated in South and Southeast Asia and is now cultivated worldwide. African cultivated rice, *Oryza glaberrima*, was domesticated in West Africa and remains locally important in some areas (Chang 1976), but it has not been a target for transgenic methods. In addition to these crops, the rice gene pool includes more than 20 wild relatives in the genus *Oryza*, and about 50 other wild species in the tribe Oryzae of the grass family (Poaceae; Vaughan 1994). Species in the genus *Oryza* include both diploids ($2n = 2x = 24$) and tetraploids ($2n = 4x = 48$), and 10 different genome types (i.e., the AA, BB, CC, BBCC, CCDD, EE, FF, GG, JJHH, and JJKK genomes) (Vaughan 1994, Ge et al. 1999). Asian cultivated rice has the diploid AA genome. *Oryza* species with different genome types have significant reproductive isolation, making them unlikely to hybridize with each other. Hybridization between species in different genera within Oryzae is also extremely difficult, even under artificial conditions such as embryo rescue in the laboratory.

Following the gene pool concept for cultivated plants (Harlan and de Wet 1971), the rice gene pool can be divided into primary, secondary, and tertiary categories according

to the evolutionary relatedness of the crop to the wild species (figure 2). Species with the AA genome have relatively high sexual compatibility, complete chromosome pairing in meiosis of F_1 interspecific hybrids, and relatively high pollen and panicle fertility of the F_1 hybrids (Naredo et al. 1997, 1998). This indicates the potential for transgenes in GM rice varieties to escape to wild relatives through cross-pollination and persist through the survival of intraspecific and interspecific hybrids. Therefore, gene flow between cultivated rice and the AA-genome species should be assessed most carefully. Many of these species grow in close proximity to the crop, in and around rice fields (either under cultivation or abandoned), ditches, canals, marshes, and riverbanks (Vaughan 1994).

The most widespread AA-genome species that hybridizes with rice is weedy rice. Weedy rice (also known as red rice and *O. sativa* f. *spontanea*), which occurs in most rice-producing regions of the world, belongs to the same biological species as the cultivated crop (*O. sativa*). Weedy rice is genetically variable, and in some cases it may have hybridized with *Oryza rufipogon* (Vaughan et al. 2003) and with cultivated rice (see below). Rice fields are the primary habitat for weedy rice, and the weed can substantially reduce crop yields (Baki et al. 2000). Weedy rice can be very difficult to control, because many of its seeds disperse before the crop is harvested and then accumulate in the soil seed bank. Also, undispersed seeds on weedy rice plants can be collected by farmers and inadvertently planted with the next generation of crop seeds. Competition with the cultivated crop and contamination of harvested seed with undesirable weedy rice grains make weedy rice a serious problem for rice growers worldwide.

Six wild rice species with the AA genome can also co-occur with the crop on different continents (figure 3). These include perennial *O. rufipogon* and annual *Oryza nivara* from Asia, perennial *Oryza longistaminata* and annual *Oryza barthii* from Africa, perennial *Oryza glumaepatula* from Latin America, and annual *Oryza meridionalis* from northern Australia and New Guinea (Vaughan 1994, Vaughan et al. 2003). All of these taxa have the potential to hybridize with the cultivated rice crop and with each other.

Extent of pollen-mediated gene flow

To understand how far rice pollen grains can travel to sire seeds on other plants, and how frequently this occurs, we must examine the reproductive biology of the crop and each type of recipient population—other cultivated rice plants, weedy rice growing within the crop, and wild rice plants that occur nearby.

General considerations. Measuring the rates at which gene flow occurs is a challenging task in any complex of wild and domesticated plants. In rice, pollen-mediated gene flow is influenced by variation in flowering times, outcrossing rates, population sizes, distances between populations, wind speed, humidity, and other factors. Several approaches can be used to estimate relative rates of pollen dispersal from crops,

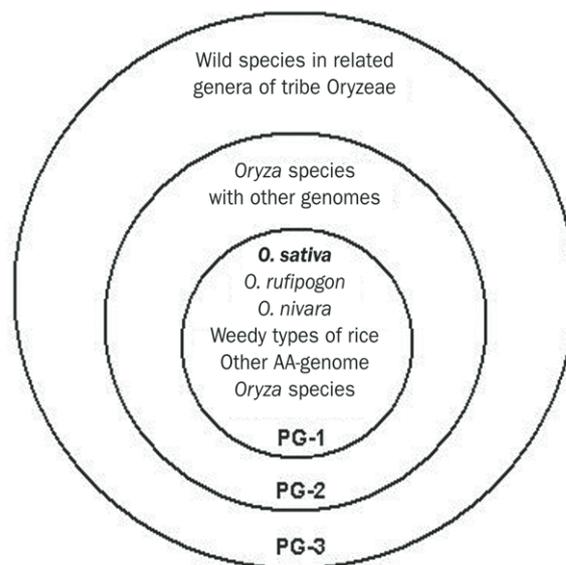


Figure 2. The gene pool of Asian cultivated rice (*Oryza sativa* L.), following the approach of Harlan and de Wet (1971). The primary gene pool (PG-1) of Asian cultivated rice encompasses its weedy types (also referred to as *Oryza sativa* f. *spontanea*), ancestral wild species (*Oryza rufipogon* and *Oryza nivara*), and other AA-genome *Oryza* species. The secondary gene pool (PG-2) comprises other non-AA-genome wild species in the genus *Oryza*, and the tertiary gene pool (PG-3) includes species in other genera within the tribe Oryzaceae in the grass family (Poaceae).

regardless of whether the crop is transgenic. Information about individual outcrossing rates and variation in flowering times is useful for evaluating the potential for hybridization. Small-scale experiments involving plants with distinct genetic markers can be used to measure gene flow between adjacent plants in a given location and year, but they may not reflect large-scale or long-term processes. Although these types of information are undoubtedly incomplete, they can be used to assess the potential for transgene escape and to develop strategies to minimize the escape of certain types of transgenes through pollen.

Seed-mediated gene flow can also be highly effective as a means of transgene dispersal, especially when seeds are traded within and between countries. Usually, rice seeds have their hulls removed before they are shipped and exported for food consumption. Therefore, in most cases, the seeds are not viable because their embryos are damaged during the milling process. But sometimes rice seeds are transported without de-hulling, including seeds that are intended for domestic seed sales. Also, viable seeds can be dispersed when the grain is threshed and dried in the open air, and when it is handled, sorted, and transported for milling. Few studies have attempted to quantify the extent of gene flow by means of seed dispersal in rice or other cultivated species. Therefore, in the

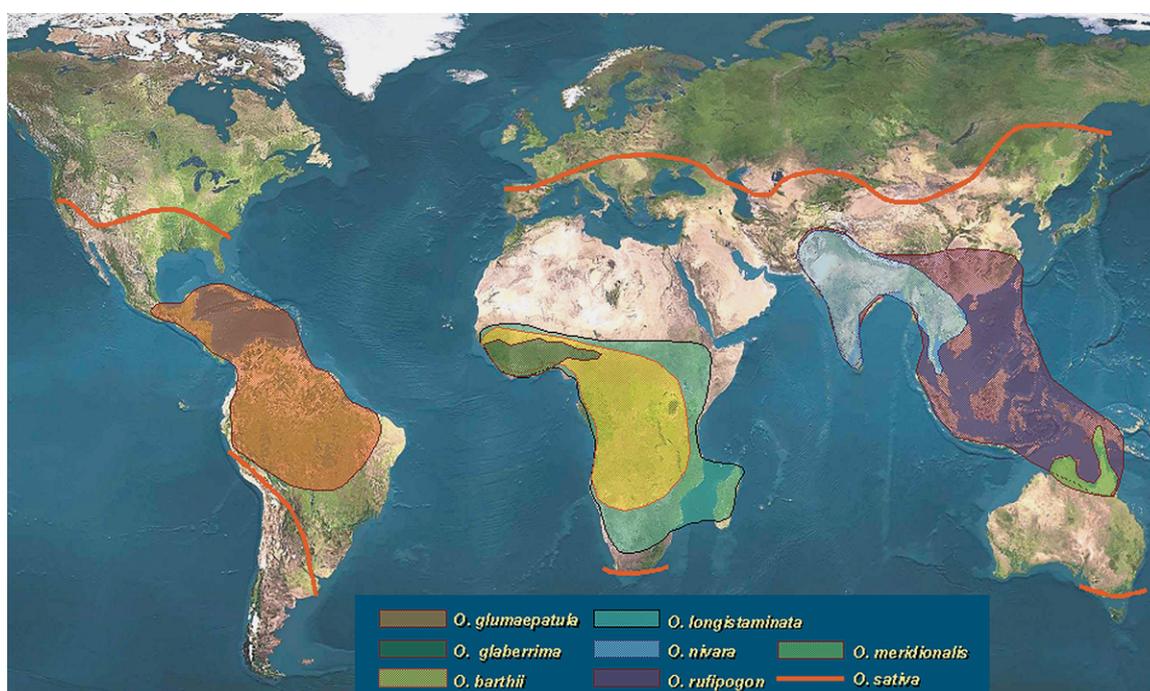


Figure 3. Global distributions of cultivated *Oryza sativa* and its sexually compatible wild relatives (genus *Oryza*) with the AA genome. Orange lines indicate the maximum range limits of cultivated and weedy rice (*O. sativa* f. *spontanea*). Modified from Lu and colleagues (2003).

following sections, we focus mainly on pollen-mediated gene flow from cultivated rice (*O. sativa*) to other rice crops, weedy rice, and other wild rice (*Oryza*) species. We begin by discussing what is known about the extent of gene flow in rice, followed by a section on the potential consequences of this process.

Crop-to-crop gene flow. The movement of transgenes from one crop field to other fields planted with non-GM varieties has the potential to increase opportunities for subsequent gene movement to weedy or wild rice populations. On a practical level, an understanding of crop-to-crop gene flow via both pollen and seeds is useful if different growers or countries want to segregate GM rice from non-GM varieties for marketing or regulatory reasons.

In general, cultivated rice is characterized by high rates of self-pollination and very little cross-pollination between adjacent plants or fields (typically less than 1 percent). Experiments in Italy showed that pollen-mediated gene flow from a transgenic, herbicide-resistant rice variety to adjacent plants of a nontransgenic counterpart was 0.05 to 0.53 percent (Messeguer et al. 2001). Modern rice cultivars are often grown near older landraces (locally adapted varieties that were domesticated and improved by traditional farmers) in Asia, and hybridization rates between these two groups also appear to be very low (Rong et al. 2004). These findings are consistent with the small distances that are recommended for isolating and maintaining the purity of cultivated rice grown in seed nurseries. In the United States, for instance, rice plants

that are grown for certified seed to be sold to farmers must be isolated from other rice varieties by only 6 meters (m) or less (Gealy et al. 2003).

Crop-to-weed gene flow. It is widely assumed that transgenes introduced into modern rice cultivars will make their way into weedy rice populations, even though the rate of cross-pollination is quite low (Gealy et al. 2003). Both taxa are primarily self-pollinated and have short-lived pollen, but low levels of crop-to-weed gene flow have been detected in many different studies. For example, gene flow from experimental plots of herbicide-resistant GM rice to weedy rice accessions ranged from 0 percent to 0.06 percent in China and southern Europe (Chen et al. 2004, Messeguer et al. 2004). Likewise, Gealy and colleagues (2003) reviewed previous studies of gene flow from cultivated to weedy rice and noted that typical rates of crop–weed hybridization were approximately 0.01 percent to 1 percent. They also noted that gene flow can occur in either direction, from crop to weed or from weed to crop (the taller stature of weedy rice can facilitate flow in the latter direction). The extent to which crop genes are passed to weedy populations is lowest when the flowering times of the crop and the weed are partially or wholly asynchronous, as occurs in some situations. However, it is also possible that the crop itself can establish feral populations (Baki et al. 2000), which could then possess transgenes. The question of whether such “de-domestication” occurs with modern cultivars has not been studied adequately, and the significance of this type of gene flow is unknown.

Another important but little-studied component of gene flow is the persistence of crop genes following crop–weed hybridization. Just as hybrid vigor is seen when inbred, cultivated lines are crossed to produce “hybrid” rice, so may weedy rice benefit from hybridizing with the crop, if this results in greater heterozygosity. In Louisiana, for example, Langevin and colleagues (1990) reported greater vigor in crop–weed hybrids than in their weedy parents, and frequencies of crop alleles in weedy rice were as high as 52 percent after only two years of contact with the crop. However, in Arkansas, first-generation hybrids between cultivated and weedy rice flowered so late that they had much lower fitness than their weedy parents (Zhang et al. 2003). Thus, the evolutionary importance of hybrid vigor in weedy rice populations appears to be variable and should be studied more broadly.

Over the course of several generations, crop genes that are strongly deleterious to weedy rice, as well as other genes that are linked to deleterious crop genes, are likely to be purged from weedy populations by natural selection and selection pressures from farmers. Conversely, linked genes that are associated with greater survival and reproduction are expected to increase in frequency following episodes of hybridization. Thus, crop genes are expected to introgress and persist in weedy rice at rates that reflect both the frequency of hybridization and the selective effects of these genes (Ellstrand 2003).

Crop-to-wild gene flow. Several studies have detected gene flow from cultivated rice to wild rice, which outcrosses to a greater degree than weedy rice (Song et al. 2003a, Chen et al. 2004). Nearly all of this work involves Asian species such as the perennial *O. rufipogon*, the annual *O. nivara*, and intermediate forms of these taxa (Oka 1988, Vaughan 1994, Majumder et al. 1997). We are not aware of any published data on experimental studies of gene flow between the crop and native wild rice species in Africa, South America, Indonesia, or Australia, although natural hybrid swarms have been studied in Africa and Latin America (Second 1982, Oka 1988, Juliano et al. 1998, Naredo et al. 1998). Here we focus on gene flow from cultivated rice to the Asian *O. rufipogon*, which we refer to as wild rice, because it is an important source of wild germplasm that has been well studied. Gene flow from the crop could also occur by means of weedy rice acting as a genetic bridge to wild species, but this avenue of gene flow has not been investigated.

Seasonality can restrict opportunities for gene flow between cultivated and wild rice. In southeastern China and Vietnam, for example, *O. rufipogon* usually flowers during the winter months, whereas *O. sativa* crops can be cultivated during two or three cycles per year in tropical and subtropical climates. Therefore, only a subset of the annual cycle of rice production is likely to overlap with the flowering period of *O. rufipogon* in these countries. Also, wild rice populations often have a protracted flowering period at each locality, whereas the domesticated crop is much more synchronized.

Song and colleagues (2003a, 2004a) examined gene flow between a rice cultivar with high pollen production and wild *O. rufipogon* in China in experimental plots. They found that the maximum frequency of gene flow to adjacent plants of *O. rufipogon* was less than 3 percent, and approximately 95 percent of the crop–wild hybrid seeds occurred on wild plants growing within 30 m of the crop. The maximum distance over which pollen flow was detected was 110 m. In summary, these studies show that low levels of crop-to-wild gene flow can occur, but only when *O. rufipogon* occurs within approximately 50 to 100 m of the crop (which can be common), and only when their flowering times overlap.

The persistence of crop genes introduced into wild rice populations is influenced by the survival and fecundity of crop–wild hybrids, and by fitness effects of specific genes from the crop. In the case of *O. rufipogon*, F₁ hybrids have lower pollen fertility and lower seed set (i.e., they produce fewer seeds) than wild genotypes (Song et al. 2004b). This partial reproductive barrier is expected to impede the rate at which crop genes introgress into wild populations, but it will not prevent introgression altogether. Longer-term studies of the persistence of crop alleles in wild rice populations are needed to evaluate the potential for introgression of transgenes.

Information gaps and conclusions about gene flow. Our review of the peer-reviewed literature reveals several major gaps in studies of gene flow between cultivated rice and its weedy and wild relatives. For example, many wild taxa have not been studied, the amount of backcrossing and introgression has not been examined adequately in any system, and little is known about how easily modern rice cultivars can revert to feral weedy rice and thereby disperse as weedy biotypes. Also, no studies of crop-to-wild gene flow have been carried out in Africa, where the perennial rice *O. longistaminata* is unusual in being a self-incompatible plant that outcrosses (Vaughan 1994). Nonetheless, it seems reasonable to conclude that the extent of pollen-mediated gene flow from cultivated rice to many of its wild and weedy relatives is quite low, on the order of 0 to 1 percent for weedy rice and 3 percent in one study of *O. rufipogon*. The distance over which cross-pollination occurs is often less than 30 m (Gealy et al. 2003, Song et al. 2003a). This is very different from other crop–wild–hybrid complexes in which weedy or wild plants outcross and hybridize extensively over distances of hundreds of meters (Ellstrand 2003).

Consequences of gene flow

Because of the high selfing (self-fertilizing) rates of rice and most of its wild relatives, this crop is sometimes regarded as a low-risk species with regard to gene flow from transgenic varieties (Stewart et al. 2003). However, if a transgene confers a strong enough fitness advantage, it could spread quickly through wild or weedy rice populations, even with very low rates of cross-pollination from the crop (Ellstrand 2003). Also, it is important to recognize that seed-mediated gene flow can be extensive in both cultivated and weedy rice. Strategies

for confining the spread of certain transgenes need to consider the dispersal and longevity of both seeds and pollen, as well as the selective advantage of specific transgenic traits.

Spread of transgenic herbicide resistance. Transgenic herbicide resistance is a trait that could easily be acquired by weedy rice. Weed control in rice fields is increasingly dependent on herbicides in both developed and developing countries, partly because older methods of hand transplanting young rice plants into flooded fields are being replaced by direct seeding techniques. This transition has resulted in worse problems with weeds, because weed seedlings can suppress the growth of rice seedlings (Baki et al. 2000). Rice fields that become heavily infested with weedy rice can become unusable, because the weed is an effective mimic of the crop and its long-lived seed bank makes it very difficult to eradicate. Thus, rice growers who can afford the cost of herbicides are eager to adopt herbicide-resistant rice varieties, even though the benefits of this strategy could be short-lived.

In the United States, three major types of herbicide-resistant rice are commercially available or nearly available, and two others have been considered for commercialization (table 1). The first type to be widely used is a nontransgenic variety that originated from chemical mutagenesis and was commercialized by BASF in the United States in 2002. Known as Clearfield rice, this variety is resistant to acetolactate synthase-inhibiting herbicides such as imidazolinone. Meanwhile, Bayer Crop Science (formerly Aventis) has received regulatory approval to commercialize LibertyLink rice, which has transgenic resistance to phosphinic acids such as glufosinate, and Monsanto has developed Roundup Ready rice, with transgenic resistance to glyphosate. So far, neither of these transgenic varieties has been used in farmers' fields. In China, several types of herbicide-resistant rice are being developed by different institutions. Some of these varieties are designed for better weed control, and others are used for maintaining seed purity in high-yielding hybrid rice production.

The three types of herbicides mentioned above—imidazolinone, glufosinate, and glyphosate—are widely used worldwide, and they have the advantage of having limited long-term persistence in the environment. Glyphosate, in particular, is an especially effective broad-spectrum herbicide that is used on hundreds of millions of hectares of cropland in many different countries. Resistance to each of these three types of herbicides is inherited as a dominant Mendelian trait that can easily spread to weedy rice by cross-pollination (Gealy et al. 2003). Although hybridization rates may be less than 1 percent, rice fields in which weedy and cultivated plants flower simultaneously are expected to produce many transgenic weed-crop hybrid seeds per hectare, because each weedy plant can produce hundreds of seeds. Also, in some cases, heterosis in first-generation weed-crop hybrids might enhance the fitness of these plants and thereby accelerate the introgression of crop alleles. If the same herbicide is used repeatedly, selection favoring transgenic, herbicide-resistant weedy rice will be very strong. Modeling studies by Madsen and col-

leagues (2002) estimated that herbicide resistance may become common in weedy rice populations within only 3 to 8 years of continuous rice cropping. Therefore, it seems extremely likely that new genes for herbicide resistance will spread to weedy rice, especially in regions where weed management is already difficult.

Many weeds of cultivated rice have evolved resistance to commonly used herbicides, so it is important to maintain the effectiveness of "environmentally friendly" herbicides such as glyphosate and glufosinate. This is such a major concern that several authors have recommended that herbicide-resistant rice should not be used widely without strict stewardship guidelines or effective biological confinement techniques, or both (Gressel 2000, Olofsdotter et al. 2000, Madsen et al. 2002). In Costa Rica, for example, smallholder farmers often rent farmland, and they do not have the option of implementing adequate integrated pest management for weedy rice (Bernal E. Valverde, Tropical Agriculture Research and Development, Alajuela, Costa Rica, personal communication, 4 September 2004). This is a region where the rapid evolution of glyphosate-resistant weedy rice could have major economic consequences for rice farmers, who rely on glyphosate for no-till rice production. These farmers might be forced to switch to tank mixes of more persistent, more expensive, and less effective herbicides, if they can afford them. Worldwide, the increased use of more persistent herbicides could have harmful effects on human health, animal health, and local ecosystems, especially in the aquatic habitats that are found in and around rice fields. Thus, the acquisition of new types of herbicide resistance by weedy rice might have both economic and environmental costs.

Effects of other fitness-related transgenes. In many cases, other transgenes are not expected to persist and spread in weedy or wild populations. If the transgenes encode traits that do not enhance the plants' survival or reproduction, the escape of these genes is unlikely to result in environmental problems, because the new genes will be very rare. Likewise, if the transgenes confer a fitness cost, in the form of reduced survival or fecundity, individuals bearing these traits will be less likely to pass them on to their progeny (Gressel 2000). Many transgenic traits related to nutritional quality and grain composition are likely to have neutral or negative effects on the fitness of weedy and wild relatives of the crop.

In contrast to these examples, the fitness of wild and weedy rice populations might be enhanced by transgenes that confer better pest resistance, greater tolerance of abiotic stresses such as drought and salinity, and enhanced yields (table 1). Depending on local conditions, these transgenes might release weedy or wild populations from ecological pressures that restrict their local abundance or limit their habitat requirements.

Several interrelated questions arise regarding the environmental effects of fitness-enhancing transgenes. First, given these genes' potential to spread and persist, their possible negative effects on nontarget species should be considered

(NRC 2002). Would the transgene or its products lead to harmful effects on beneficial insects, wildlife, or other species, and how would these effects compare with possible harm resulting from conventional agricultural practices (e.g., existing uses of pesticides)? How might pharmaceutical-producing rice plants affect nontarget species? Some types of transgenes may provide environmental benefits for nontarget species. In China, for example, the use of insect-resistant *Bt* (*Bacillus thuringiensis*) rice could be an improvement over current insecticide treatments in terms of its expected effects on nontarget species (Bashir et al. 2004, High et al. 2004).

Second, for a given country and region, it is important to ask whether the receiving population is already a weed or has the potential to become more invasive by acquiring specific transgenic traits. Annual weedy rice species are clearly significant weeds worldwide, so they should be studied carefully in this regard. Perennial wild rice species appear to be less of a problem for agriculture, although they sometimes reduce crop yields in parts of Africa and Asia (Vaughan 1994, Holm et al. 1997, Baki et al. 2000). Also, natural hybrids between perennial wild rice species and cultivated rice may cause weed problems. For example, weedy types of *O. glumaepatula* are thought to originate from hybridization between this Latin American species and cultivated rice (Juliano et al. 1998, Naredo et al. 1998).

Some perennial rice species might become more invasive in natural habitats, such as brackish and saline marshes (for example, by acquiring transgenes that confer salt tolerance and faster growth rates), but the extent to which these taxa are considered weedy or potentially weedy varies a great deal geographically. For example, *O. rufipogon* has the potential to be an invasive species in the United States (Vandiver et al. 1992), where it is listed as a noxious weed, but it is becoming very rare within parts of its native range. In China, at the northern end of its range, *O. rufipogon* is threatened with local extinction (Song et al. 2003b). In the Mekong Delta of Vietnam, however, *O. rufipogon* is fairly abundant. This species can be a weed in cultivated fields, and it is common along the margins of rivers and canal banks, where it is sometimes harvested for animal feed.

A third major question about the consequences of gene flow from transgenic rice relates to the magnitude of the fitness benefit from transgenes, and whether this benefit affects population dynamics. Virtually nothing is known about the extent to which insect and disease pressures regulate populations of weedy or wild rice, and ecological studies of these populations are needed to help address this question. In wild sunflower, for example, a single *Bt* gene resulted in less damage from insect larvae, and a large boost in fecundity, in field-grown experimental plants in Nebraska (Snow et al. 2003). Likewise, nothing is known about how novel genes for increased crop yield will affect the growth and fecundity of weedy or wild rice. Details about the nature and efficacy of transgenes that confer the trait for increased yield are not available to the public, because they represent confidential business information (table 1). This makes it impossible for independent scientists

to evaluate whether gene flow from such crops would pose a biosafety hazard by causing weedy relatives of the crop to become more prolific.

A fourth question relates to the concern that targeted insects will quickly evolve resistance to transgenes such as *Bt*, which offers several environmental and human health advantages over the use of broad-spectrum insecticides (NRC 2002). In the United States, farmers who plant *Bt* crops are required to use a "high dose/refuge" plan for delaying the evolution of *Bt* resistance in target insect pests (NRC 2002). However, this practice will be difficult to achieve in some countries. The frequencies of *Bt* transgenes and the effects they have on insects that feed on weedy and wild rice might jeopardize the success of resistance management efforts. In this case, the environmental concern is that a valuable alternative to chemical pesticide applications could be lost prematurely.

In general, it seems likely that most fitness-enhancing traits could not spread to weedy populations as quickly as the genes for herbicide resistance could in populations that are frequently exposed to the herbicide in question. As noted above, self-pollination restricts the rates at which transgenes enter weedy and wild rice populations. However, once this has occurred, self-pollinating plants with greater fitness can increase the numbers of transgenic progeny in subsequent generations. In perennials such as *O. rufipogon*, *O. longistaminata*, and *O. glumaepatula*, vegetative propagation and competitive displacement among clones could also promote transgene persistence. Gradually, fitness-enhancing transgenes may be able to spread to weedy and wild relatives of the crop and perhaps accumulate as a suite of newly acquired traits. Therefore, if certain transgenes can enhance the fitness of wild or weedy rice species, it is important to evaluate (a) the potential for creating more invasive weeds; (b) the possible harm to nontarget species, such as beneficial insects; and (c) the possible effects of gene flow on the long-term durability of *Bt* and glyphosate-resistant crops (NRC 2002, Snow et al. 2005).

Effects on the genetic diversity of wild germplasm. Wild relatives of crop plants are widely viewed as valuable sources of genetic diversity for future breeding (Vaughan 1994, Ellstrand 2003). Even if the crop's wild relatives are somewhat weedy, germplasm experts believe that these reservoirs of diversity should be protected from population extinction and genetic "swamping," which results from a heavy influx of crop genes. To some people, the mere presence of artificial transgenes in the wild germplasm of crop relatives represents a form of contamination, or "genetic pollution." On a purely scientific basis, however, it is important to focus on whether native genetic diversity could be displaced by evolutionary processes that involve transgenes. We propose that this outcome is unlikely, for the reasons stated below.

Two scenarios for negative effects of transgenes on genetic diversity have been suggested. First, it is theoretically possible that strong selection for fitness-enhancing transgenes could generate "selective sweeps," in which portions of the crop genome that are linked to these transgenes displace corre-

sponding portions of wild genomes (Ellstrand 2003, Gepts and Papa 2003). This process is expected to be more common in self-pollinating species than in obligate outcrossers, which have greater opportunities for mixing and diluting crop alleles during sexual reproduction. Also, selective sweeps could be favored by clonal reproduction, which might allow more vigorous transgenic crop-wild hybrids to outcompete non-transgenic plants on a local scale. These conditions are met in rice, but the potential for rapid selective sweeps seems remote, because (a) few transgenes seem likely to confer fitness benefits that are strong enough to lead to selective sweeps in wild populations (i.e., populations outside farmers' fields), and (b) the extent of pollen-mediated gene flow is typically very low.

A second scenario for harmful effects on wild germplasm focuses on local extinction. In some situations, a large influx of fitness-reducing transgenes could contribute to population declines of small, isolated populations of wild plants that occur near the crop (Haygood et al. 2003). The movement of transgenes that cause population declines seems extremely unlikely in most wild relatives of rice, because of low rates of hybridization, large population sizes, and occasional seed or rhizome dispersal among populations. In populations of, say, 100 individuals or more, we expect that frequencies of fitness-reducing transgenes would diminish as a result of the purifying force of natural selection. Therefore, current information suggests that gene flow from transgenic rice will not threaten the genetic diversity of the crop's wild and weedy relatives to a greater extent than gene flow from conventional varieties (Ellstrand 2003, Gepts and Papa 2003).

Conclusions

Ideally, the commercial release of GM rice varieties should be preceded by scientific research on environmental consequences. However, even though plans for commercialization are imminent, the ecological effects of gene flow have not been examined adequately, especially in tropical and subtropical countries. At this point, it is not clear whether low frequencies of gene flow to wild and weedy relatives of the crop could result in unwanted and possibly irreversible environmental effects. In each of the world's major rice-growing countries, information about the geographic distribution, population ecology, and reproductive biology of sympatric wild relatives of the crop will be useful for evaluating the ecological effects of new transgenic traits. This knowledge will assist in promoting the further development of transgenic biotechnology and the safe use of promising GM rice varieties worldwide. For example, it is especially important to gain a better understanding of the consequences of gene flow from transgenic rice to weedy rice populations. Transgenic traits that have the potential to enhance the fitness of the crop's relatives should be assessed with extra caution, because these genes are expected to increase in frequency following episodes of crop-wild hybridization, and because it is difficult to predict their ecological consequences.

In conclusion, it is worth emphasizing that gene flow is a natural process that happens inevitably under natural conditions. On the basis of current ecological and evolutionary knowledge, we believe that the dispersal of many types of transgenes from rice will not be harmful to the environment. Transgenes that do not have significant natural selective advantages should cause limited environmental impact, if any. On the other hand, transgenes that lead to greater abundances of weedy or wild rice could pose environmental problems, as described above. Before permitting the commercial release of new types of transgenic rice, policymakers need to be able to weigh the net environmental benefits and risks of such transgenes against other factors, such as the anticipated effects of transgenic varieties on human health, local prosperity, and trade. Environmental assessments that are scientifically rigorous and publicly accessible are essential for the long-term success of this important technology.

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