Current knowledge of gene flow in plants: implications for transgene flow

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Plant evolutionary biologists' view of gene flow and hybridization has undergone a revolution. Twenty-five years ago, both were considered rare and largely inconsequential. Now gene flow and hybridization are known to be idiosyncratic, varying with the specific populations involved. Gene flow typically occurs at evolutionarily significant rates and at significant distances. Spontaneous hybridization occasionally has important applied consequences, such as stimulating the evolution of more aggressive invasives and increasing the extinction risk for rare species. The same problems have occurred for spontaneous hybridization between crops and their wild relatives. These new data have implications for transgenic crops: (i) for most crops, gene flow can act to introduce engineered genes into wild populations; (ii) depending on the specific engineered gene(s) and populations involved, gene flow may have the same negative impacts as those observed for traditionally improved crops; (iii) gene flow's idiosyncratic nature may frustrate management and monitoring attempts; and (iv) intercrop transgene flow, although rarely discussed, is equally worthy of study.

Keywords: crop-to-wild gene flow; introgression of engineered genes; transgene flow; evolution of invasiveness; extinction by hybridization

1. INTRODUCTION

In the beginning, it was simple. The Neo-Darwinists explained evolutionary biology in a few broad strokes: mutation supplied the genetic variation for other evolutionary forces to act upon. Natural selection moulded adaptations out of that raw variation. Bonds of gene flow (also known as 'migration') held species together. Any differences between populations that could not be explained by locally selected adapations must have evolved by genetic drift (Stebbins 1950; Dobzhansky 1951; Mayr 1963).

According to the Neo-Darwinist view of evolutionary biology in the mid-1900s, natural selection held centre stage, but gene flow and its interspecific counterpart, hybridization, played major supporting parts. Gene flow's perceived role was to deliver new adaptive variation to the far reaches of a species' range. Likewise, hybridization and subsequent introgression delivered useful variation for continued adaptive gains (Anderson 1949; Stebbins 1950). Both gene flow and hybridization were thought to be common and important mechanisms for Darwinian change.

Around 1970, two things happened that caused a paradigm shift. First, experimental estimates of gene flow in both animals and plants showed it to be much more restricted than previously thought (Ehrlich & Raven 1969; Levin & Kerster 1974). Likewise, the view shifted regarding hybridization, from considering it a common and creative evolutionary phenomenon to an infrequent one rarely yielding more than evolutionary dead-ends (see

references in Arnold *et al.* (1999)). Second, increasing importance was attributed to natural selection as the only major component of evolution. Indeed, at that time natural selection and adaptation were introduced as theoretical components to other fields. The explanatory power of the adaptive context enriched the development of both theory and the interpretation of pre-existing data, giving rise to 'evolutionary ecology' and 'sociobiology' (Pianka 1974; Wilson 1975). Interestingly, the other three evolutionary forces did not transfer as readily. But a growing number of theoretical studies revealed that if gene flow and hybridization were as common as believed, then they would deliver maladaptive variation just as easily as adaptive variation and thereby constrain adaptive evolution (Antonovics 1968), challenging primacy of selection as the central evolutionary force.

Therefore, the role of gene flow was re-evaluated, especially in plants. By the early 1980s, dozens of experimental studies had shown that the dispersal of both pollen and seed from individual plants was typically highly skewed toward the source (Levin & Kerster 1974; Levin 1981). A typical dispersal curve is given in figure 1 from the data of Levin (1981) who progeny-tested wild *Phlox drummondii* growing around a core of 100 introduced plants of a cultivar of the same species that were homozygous for a dominant corolla lobe character. Nearly half of the successful pollinations recorded occurred at 1 m from the source population.

Despite the fact that such dispersal curves were also leptokurtic (that is, with a longer tail than expected from a normal distribution), the nearly universal conclusion was that gene flow in plants was highly restricted, rare, and of little evolutionary significance. And, of course, if

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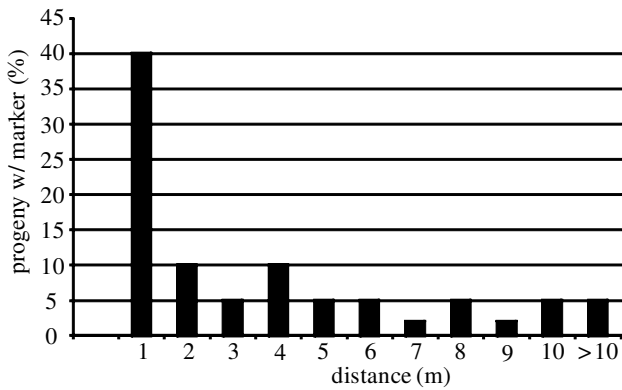DOI 10.1098/rstb.2003.1299

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Figure 1. An example of experimental gene-flow data following a dominant marker from central source plants to surrounding sink plants whose seeds were subsequently progeny tested. (Details in Levin 1981.)

intraspecific gene flow was restricted, then hybridization and introgression—which have to surmount the additional barriers of reproductive isolation—fell nearly into the category of an evolutionary oddity.

Thus, the new paradigm emerging around 1970 was even simpler than that of the Neo-Darwinists: mutation supplied the genetic variation for selection to act upon. Natural selection moulded adaptations out of that raw variation and held species together. Any differences between populations could be explained by locally selected adaptive differences (Ehrlich & Raven 1969). What about the other forces? Regarding gene flow, Levin (1981, p. 233) nicely summarized the sentiment of the time: 'Even if gene flow distances were twice as large as we now think, the spatial scale of gene dispersal: (1) is still small enough to allow substantial differentiation over short distances with moderate selective differentials, and (2) is too small to be a major cohesive force within a species.' Soon after, he wrote, 'the immigration rate is likely to be much less than 1%' (Levin 1984, p. 243).

2. TWENTY YEARS OF GENE-FLOW RESEARCH

Data challenging and eventually rejecting the hypothesis that gene flow in plants had no evolutionary significance came from scientists who were looking for something else. By the mid-1980s, several plant population geneticists had independently created 'paternity'-type analyses of seeds to assign a paternal contribution. The goals of such research projects included the measurement of gender, fitness, multiple paternity, pollen contamination of seed orchards and intra-population dispersal. But most plant paternity studies also ended up identifying a fraction of seeds that could not be assigned local fathers, that is, seeds sired by plants in another population—the result of gene flow by pollen. The level of gene flow was found to be variable, but often evolutionarily significant.

Some representative studies are listed in table 1. I have calculated some crude estimates of the number of migrants per generation (N_m) from these studies. First, the estimated gene-flow rate (m) must be halved since a gamete is a haploid immigrant. Next, the rate is multiplied by the number of individuals in the target population (N). Although the seed pool should be included, it is virtually

never known and is not accounted for in this analysis. Also, as N_m is a per-generation estimate, these per-year data should be converted to that estimate. In table 1, the values are uncorrected because it is difficult to judge how many interpopulation hybrids would enter the next generation. The first omission, plus the fact that seed immigration rates are unknown, suggests that this procedure may underestimate N_m.

These estimated gene-flow and migration rates are sometimes high enough to be evolutionarily significant in terms of counteracting the effects of drift (i.e. $N_m > 1$) and moderate selection ($m > 5\%$) (see Ellstrand (1992) for a more thorough discussion). How could it be that the prior method of measuring dispersal from a single source so underestimated the rate of interpopulation mating? Regarding this approach, Grant (1985, p. 30) observed, 'long-range dispersal events are excluded by the methods used in quantitative dispersal studies'; that is, measuring dispersal from a source almost always truncates the actual dispersal curve because it is impossible to know whether pollen or seed disperse successfully beyond the most distant collection point.

But the new data did not return plant evolutionary biology to the Neo-Darwinist view of uniformly high rates of gene flow. Gene flow was clearly much more variable than imagined by either of the prior schools. It is clear from table 1 than gene flow varies strikingly between species. But as paternity studies estimated gene-flow rates for different populations of the same species, it became clear that gene flow could vary tremendously within species as well. For example, paternity studies in orchards and natural stands of Douglas fir (*Pseudotsuga menziesii*) measured interpopulation mating rates from as low as 0.2% to as high as 52% (Neale 1983; El-Kassaby & Ritland 1986; Adams & Birkes 1990). Natural and experimental populations of wild radish (*Raphanus sativus*) isolated by 100–1000 m were found to have rates varying from 0 to 100% (Ellstrand *et al*. 1989; Devlin & Ellstrand 1990; Goodell *et al*. 1997). Overall, the new gene-flow estimates are generally so high that Rieseberg & Burke (2001) have suggested that gene flow of advantageous alleles might act as a cohesive force that integrates species, just as the Neo-Darwinists predicted.

Likewise, hybridization and introgression have been shown to vary considerably with the specific populations involved (Meyn & Emboden 1987; other examples in Rieseberg & Carney 1998). Renewed interest in hybridization and introgression, coupled with the advent of molecular genetic markers, has resulted in a new appreciation of their evolutionary and applied significance. The role of hybridization as a stimulus for speciation has received new recognition (Abbott 1992; Rieseberg 1997). Similarly, introgression has been shown to occur sometimes at rates and distances much greater than anticipated from morphological data, especially in the case of cytoplasmic introgression (Rieseberg & Soltis 1991; Rieseberg & Ellstrand 1993; Rieseberg & Carney 1998).

Spontaneous hybridization is now recognized occasionally to have important applied consequences as well. Perhaps the most notorious example is the salt marsh grass, *Spartina anglica*, in the British Isles (Gray *et al*. 1991; Thompson 1991). Britain's native salt marsh grass is *Spartina maritima*. *Spartina alterniflora* of the New World

Table 1. *Some gene-flow rate and migrant number estimates from paternity studies.* (Adapted from Ellstrand 1992.)

species (reference)	physical isolation (m)	estimated interpopulation mating rate (%)	estimated N_m (no. of migrants) (see § 2)
Cynosurus cristatus (Ennos & Dodson 1987)	'well isolated'	0.4	1.9
Gleditsia triacanthos (Hamrick & Schnabel 1985)	200	6.0	1.5
Picea glauca I (Schoen & Stewart 1987)	1000	0.1	0.3
Picea glauca II (Schoen & Stewart 1987)	1000	0.7	2.3
Pinus taeda (Friedman & Adams 1985)	122	36.0	54.0
Pseudotsuga menziesii (El-Kassaby & Ritland 1986)	8	0.2	1.3
Raphanus sativus I (Devlin & Ellstrand 1990)	150	6.3	1.3
Raphanus sativus II (Devlin & Ellstrand 1990)	150	7.0	1.4
Tachigali versicolor (Hamrick & Loveless 1989)	500	26.0	0.8

was introduced at about the mid-1800s. The two species hybridized; their sterile hybrid, *Spartina x townsendii*, was first identified in 1879. Capable of vegetative reproduction by rhizomes, *S. x townsendii* began to spread. By 1892, the seed fertile species, *S. anglica,* evolved from the sterile hybrid by chromosome doubling. The appearance of this new species is more than an academic curiosity. *S. anglica* has become a spectacularly successful invasive species, now occupying 'approximately 10 000 hectares along the coast of Britain' (Thompson 1991, p. 394). Despite its recent evolution, this species has radically altered the ecology of Britain's coasts, invading the open intertidal flats, replacing more diverse native plant communities, altering succession and limiting the food supply of birds that forage in those habitats. The example is not an isolated one. It is now clear that hybridization appears to have played a creative evolutionary part in the evolution of a remarkable number of invasive plants (Ellstrand & Schierenbeck 2000).

In addition to its creative role, it is now known that hybridization can also have a destructive role as a factor in plant extinction (Levin *et al.* 1996). The Catalina Island mountain mahogany, *Cercocarpus traskiae*, is an illustration. This endemic is native to a single gully on an island off the coast of California (33°23′ N, 118°25′ W). It hybridizes with the more common and widespread species *Cercocarpus betuloides*. Since the discovery of the island endemic, the adult population size has plunged from more than 40 to 11. A few of these appeared to be hybrids. DNA and isozyme analysis revealed that almost half of the total reproductive population, five adults, are of hybrid origin, as well as several seedlings (Rieseberg & Gerber 1995). Clearly, if future hybridization occurs, it will rapidly send the species to extinction (Wolf *et al.* 2001).

3. CROP-TO-WILD GENE FLOW HAPPENS

You might think that spontaneous gene flow from crops to wild populations would be a popular research topic for plant evolutionists. Crops are among the best studied of plant species, and as many wild crop relatives are important weeds or important germplasm sources (Smartt & Simmonds 1995), they are also better studied than most species. Potential research questions are obvious: what reproductive isolation barriers have evolved to prevent gene flow from wild plants swamping out the gains of domestication? What is the relative fitness of their

hybrids in field conditions? Have wild populations collected 'heirloom' alleles from past varieties?

But, until the last decade, hybridization between crops and their wild relatives received scant attention from plant evolutionists. One likely explanation of this is that the research field falls uncomfortably between 'basic' and 'applied' research (Ellstrand 2002). Unsurprisingly, those daring scientists who published work on crop-to-wild gene flow before the advent of transgenics are among the bright lights of their fields: for example, E. Anderson, H. Baker, S. Barrett, J. M. J. De Wet, J. Harlan and C. Heiser. These scientists saw domesticated plants and their wild, sometimes weedy, relatives as actively evolving complexes, joined through both shared ancestry and frequent hybridization (De Wet & Harlan 1975), much like what 'basic' plant evolutionists call a 'syngameon' (Grant 1981).

Attitudes about crop-to-wild gene flow have paralleled those about plants in general, as detailed already. At the time of the creation of the first transgenic plants, most plant evolutionists and geneticists considered natural hybridization to be rare and largely unimportant. Naturally, the same would hold true for crops and their wild relatives. That attitude extended to applied plant scientists, reinforced by the frustrations that some plant breeders encountered in their attempts to make wide crosses. This now outdated view has persisted in some quarters. As recently as 2000, the Director of Biotechnology at the University of California Davis wrote, 'Breeders have found that, with rare exceptions, crops do not successfully crossbreed with other plants in the environment, especially in crop-growing regions' (McGloughlin 2000, p. A39).

But as the 1980s progressed, the realization that engineered genes could move from crops to wild populations brought attention to this neglected field. Perhaps the first to address the issue were two Calgene scientists (Goodman & Newell 1985) who wrote 'The sexual transfer of genes to weedy species to create a more persistent weed is probably the greatest environmental risk of planting a new variety of crop species'.

Since then, research activity on crop-to-wild gene flow has grown with increasing vigour. Much of the effort has been to address the question of whether domesticated plants are capable of spontaneously mating with wild relatives under field conditions. Some of the work has been descriptive, documenting the presence of crop-specific

Table 2. *Some experimental estimates of domesticated-to-wild gene-flow rates.*
(Adapted from Ellstrand 2003.)

cultigen	scientific name	wild relative	hybridization rate (%)	range of distances (m)	maximum distance at which gene flow was detected (m)	citation
bread wheat	*Triticum aestivum*	*Aegilops cylindrica*	1–7	intermingled	n/a	Guadagnuolo *et al.* (2001)
foxtail millet	*Setaria italica*	*Setaria verticilliata*	0.50	0.4	0.4	Till-Bottraud *et al.* (1992)
pearl millet	*Pennisetum glaucum*	*Pennisetum sieberanum*	39	intermingled	n/a	Renno *et al.* (1997)
potato	*Solanum tuberosum*	*Solanum nigrum*	0	0–20	n/a	McPartlan & Dale (1994)
radish	*Raphanus sativus*	same species	0–100	1–1000	1000	Klinger *et al.* (1991)
rapeseed	*Brassica napus*	*Sinapis arvensis*	0	intermingled	n/a	Lefol *et al.* (1996)
sorghum	*Sorghum bicolor*	*Sorghum halapense*	0–100	0.5–100	100	Arriola & Ellstrand (1996)
squash	*Cucurbia pepo*	*Cucurbia texana*	5	1300	1300	Kirkpatrick & Wilson (1988)
sugar beet	*Beta vulgaris*	same species	1	0–210	210	Vigouroux *et al.* (1999)
sunflower	*Helianthus annuus*	same species	0–27	3–1000	1000	Arias & Rieseberg (1994)

alleles in wild populations that grow near cultivated relatives. For example, Bartsch & Ellstrand (1999) examined the allozymes of the weed beet *Beta macrocarpa* from several populations. They detected an allele specific to sugar beet in *ca.* 2% of the plants growing in California's Imperial Valley, an area of sugar beet (*Beta vulgaris*) production, but they did not find that allele in any *Beta macrocarpa* populations growing allopatric from sugar beet. Clearly, that allele had introgressed from the crop to the weed.

Furthermore, many experiments have been performed to measure spontaneous hybridization rates between crops and cross-compatible wild relatives; representative examples are listed in table 2. Typically, these experiments involve a stand of crop plants surrounded by synthetic populations of a wild relative. The plants are selected so that the crop bears an allele that is absent in the wild plants (most studies use a natural marker, not a transgene). Progeny testing of seed harvested from the wild plants identifies those that bear the allele specific to the domesticate. That fraction of progeny is the estimate of the hybridization rate. Those rates vary considerably, but they also reveal that, depending on the system, plant gene flow can occur at remarkable distances and at remarkable rates (table 2).

Generally, for most crops, spontaneous hybridization with wild relatives appears to be the rule. A recent review showed that 12 out of the world's 13 most important crops are known to engage in such hybridization (Ellstrand *et al.* 2002). Indeed, there is now substantial evidence that at least 48 cultivated plants mate with one or more wild relatives somewhere in the world (table 3).

And, just as in wholly natural systems, hybridization occasionally has had significant consequences. Over the past few decades, weed beets—evolving from hybrids between sugar beet and wild sea beet—have resulted in millions of dollars of damage to northern Europe's sugar beet industry (Boudry *et al.* 1993; Desplanque *et al.* 1999). Gene flow from crops into wild populations has been implicated in several other cases of weed evolution, including new invasive species (Ellstrand & Schierenbeck 2000). Also, gene flow from crops has been implicated

in the increased likelihood of extinction of wild relatives. Natural hybridization with cultivated rice has been implicated in the near extinction of the endemic Taiwanese taxon, *Oryza rufipogon* ssp. *formosana* (Kiang *et al.* 1979). Collections of this wild rice over the past century show a progressive shift towards characters of the cultivated species and a coincidental decrease in fertility of seed and pollen. By 1979, wild rice in Taiwan was on the verge of extinction. Indeed, throughout Asia, typical specimens of other subspecies of *O. rufipogon* and the wild *Oryza nivara* are now rarely found owing to extensive hybridization with the crop (Chang 1995). Hybridization with domesticated species has been thought to play a part in the extinction of the wild relatives of several other crops (Small 1984).

4. TRANSGENE FLOW?

Out of the environmental concerns voiced about genetically engineered plants, those associated with the escape of engineered genes (also known as 'transgenes') into the populations of wild relatives have received the most attention. Almost every general treatment of the environmental impacts of plant biotechnology gives some consideration to gene flow (Colwell *et al.* 1985; Tiedje *et al.* 1989; NRC 1989, 2000, 2002; Hails 2000; McHughen 2000; Pretty 2001; Dale *et al.* 2002). What lessons can be applied to transgene flow from what we already know about gene flow in plants and, more specifically, from what we already know about gene flow from crops to their wild relatives?

(a) *Lesson 1: it is not unusual for crops to mate with their wild relatives*

If a transgenic crop is released in regions where wild relatives grow, we would expect that spontaneous hybridization will occur unless the engineered plants are specially designed to limit gene flow (cf. Gressel 1999; Daniell 2002). And unless those transgenes are deleterious, they will generally persist and introgress into the natural populations.

Table 3. Cultivated plants for which there is genetic evidence of spontaneous hybridization with wild relatives. (Adapted from Ellstrand 2003.)

cultigen	scientific name
alfalfa	*Medicago sativa*
apple	*Malus x domestica*
avocado	*Persea americana*
banana	*Musa acuminata*
bean, common	*Phaseolus vulgaris*
beet	*Beta vulgaris*
bentgrass, creeping	*Agrostis stolonifera*
cacao	*Theobroma cacao*
cane, sugar	*Saccharum officinarum*
cassava	*Manihot esculenta*
cocona	*Solanum sessiliflorum*
coffee, arabica	*Coffea arabica*
cotton	*Gossypium barbadense*
cotton	*Gossypium hirsutum*
elm, Siberian	*Ulmus pumila*
fescue, tall	*Festuca pratensis*
gourd	*Cucurbia pepo*
grapes	*Vitis vinifera*
juniper	*Juniperus chinensis*
lettuce	*Lactuca sativa*
maize	*Zea mays* ssp. *mays*
millet, foxtail	*Setaria italica*
millet, pearl	*Pennisetum glaucum*
mushroom, button	*Agaricus bisporus*
oats	*Avena sativa*
poplar, hybrid	*Populus trichocarpa x P. deltoides*
potato	*Solanum stenotomum*
potato	*Solanum tuberosum*
quinoa	*Chenopodium quinoa*
radish	*Raphanus sativus*
rape, swede	*Brassica napus*
rape, turnip	*Brassica campestris*
raspberry	*Rubus idaeus*
rhododendron, catawba	*Rhododendron catawbiense*
rice	*Oryza glaberrima*
rice	*Oryza sativa*
rye	*Secale cereale*
ryegrass	*Lolium perenne*
salsify	*Tragopogon porrifolius*
sorghum	*Sorghum bicolour bicolour*
soybean	*Glycine max*
squash	*Cucurbia pepo*
strawberry	*Fragaria x ananassa*
sunflower	*Helianthus annuus*
walnut	*Juglans regia*
watermelon	*Citrullus lanatus*
wheat, bread	*Triticum aestivum*
wheat, durum	*Triticum turgidum durum*

(b) *Lesson 2: gene flow, in itself, does not necessarily create problems*

Descriptive studies have demonstrated that crop alleles have introgressed into natural populations but, in many cases, appear to have no more impact than enhancing local genetic diversity in the wild populations (Ellstrand 2003).

(c) *Lesson 3: natural hybridization occasionally results in problems in terms of increased weediness or invasiveness*

We would expect the same from transgenic crops. Clearly, the probability of problems owing to gene flow

from any individual cultivar is extremely low, but when those problems are realized, they can be very significant. Whether transgenic crops are more or less likely to create gene-flow problems depends in part on the phenotypes conferred by the transgenes (Ellstrand & Hoffman 1990). But it is challenging to identify which crop genes may push a wild species over the edge to invasiveness. For example, although gene flow has been introducing pest-resistance alleles from crops to wild relatives for generations, there are no known examples of increased invasiveness owing to introgression of those alleles (Traynor & Westwood 1999).

Although transgene introgression into wild populations is not yet known to have created any new or more difficult weeds, a recent incident in Alberta involving gene flow among herbicide resistant crop varieties may give a taste of things to come. Spontaneous hybridization occurred between three different varieties (two transgenic, one not) of canola that had been planted near to one another in 1997 and 1998, each resistant to a different herbicide (glufosinate, imidazolinone and glyphosate; Hall *et al.* 2000). As a result of the hybridization, by late 1998 volunteers were resistant to multiple herbicides. The resistance alleles moved rapidly. Scientists studying the volunteers reported, 'a single triple-resistant individual was located more than 550 m from the putative pollen source 17 months after seeding' (Hall *et al.* 2000). Even though multiple-resistant *Brassica napus* volunteers are typically in low frequency, their presence warrants more complicated weed management.

(d) *Lesson 4: natural hybridization occasionally results in negative impacts in terms of increased extinction risk to wild relatives*

We would expect the same from transgenic crops. Again, the probability of problems owing to gene flow from any individual cultivar is extremely low. Nevertheless, if a new allele, transgene or not, causes an increase in crop gene flow into a wild population, then that wild population has an increased chance of extinction by hybridization.

(e) *Lesson 5: Gene flow varies tremendously, both between species and within species*

Various proposals have been offered to limit transgene flow through ecological methods, such as surrounding the transgenic crop with barren zones or barrier crops to reduce pollen flow (reviewed by Kareiva & Marvier 2000) or through genetic methods, such as plastid transformation to prevent transgene transmission via pollen (Daniell 2002) or 'tandem constructs' to handicap the fitness of transgenic hybrids (Gressel 1999). Given that gene flow varies with species, population, genotypes, environments, between seasons and within seasons, it is clear that the efficacy of such methods must be tested under a variety of circumstances.

This lesson also has consequences for the monitoring for transgene flow. Monitoring transgenes has been proposed as a way to measure their possible environmental impacts and to serve as a warning system for deleterious effects (NRC 2002). If gene flow were predictable, it might be easy to target when, where and how to monitor. Given the idiosyncratic nature of gene flow, and given that a tiny amount of gene flow is sufficient to establish an

allele in a population, it will be difficult to create an effective monitoring programme (see also Marvier *et al.* 1999).

(f) *Lesson 6: typically, intraspecific gene flow occurs at surprisingly high rates and over surprisingly high distances*

From tables 1 and 2 it is clear that, for adjacent populations, conspecific gene flow of more than 10% is not uncommon. For outcrossing species, it is not unusual for conspecific gene flow at a thousand metres' isolation to be 1%, which is orders of magnitude higher than the mutation rate. But concerns about transgene flow have largely neglected crop-to-crop gene flow. Two recent events have demonstrated that intercrop gene flow has already delivered transgenes into plants for which they were not intended and that gene flow may have environmental, economic, agronomic or social impacts.

(i) As mentioned, spontaneous sequential cross-pollination between three varieties (two transgenic) of canola resistant to different herbicides resulted in the evolution of multiple-herbicide-resistant volunteers (Hall *et al.* 2000). Whereas other herbicides are available for controlling these new weeds, the range of options has now been reduced, especially for farmers who want to remove volunteer canola from other crops resistant to the three herbicides in question.

(ii) The identification of transgenes in remote Mexican maize landraces (Alvarez Morales 2002) after years of that country's moratorium on transgenic maize has raised discussion about whether post-commercialization transgene containment is likely or even possible (Hodgson 2002).

In retrospect, one could have anticipated that the initial detection of unintended transgene flow would be associated with hybridization among crops rather than between a crop and a wild relative. In addition to the foregoing events, the unintentional spread of transgenes as a result of intercrop gene flow may have other important consequences, as follows.

(i) The pollination of seed crops intended for human or animal consumption by plants transformed to create industrial biochemicals may pose human or animal food-safety issues (Ellstrand 2001).

(ii) The pollination of crops intentionally grown for organic produce may have their certification jeopardized if that produce contains seeds or seed products resulting from seeds that were sired by transgenic plants (NRC 2002).

(iii) Seed from plants that have been unintentionally pollinated by transgenic plants may serve as 'genetic bridges' that transfer transgenes to other varieties or wild relatives (NRC 2002).

While there is certainly no reason to abandon research on crop-to-wild gene flow, the data and skills accumulated regarding that question may prove helpful in addressing the simpler, but perhaps more urgent issues of transgene flow among crops.

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Discussion

S. C. H. Barrett (*Department of Botany, University of Toronto, Toronto, Canada*).

(i) The data that you have shown us on measuring gene flow largely come from small experimental plots in which a high percentage of plants are on the periphery of the plots. Moreover, most of the populations studied were close enough that many investigators might not really consider these as distinct populations since they are less than 1 km apart. Do you think that this may inflate the levels of gene flow that are occurring in other situations—such as large populations separated by more than 1 km?

(ii) You provided a gene-flow estimate of 100% for *Raphanus*; I cannot see how this occurs. What is wrong with intra-population pollen? Why is all the seed sired from pollen from outside of the population?

(iii) Do you think that seed spillage from vehicles may be a more important source of gene movement across the landscape?

N. C. Ellstrand.

(i) Yes these estimates might overestimate gene-flow estimates for the types of population you describe.
(ii) We measured 100% gene flow in experimental plots that were typically very small (fewer than 10 individuals) and close to large populations.
(iii) Yes, I agree that seed spillage is likely to be an important source of seed.

S. Linington (*Royal Botanic Gardens, Kew, Ardingly, West Sussex, UK*). What do you predict is the long-term future of on-farm conservation of landraces? There is an increasing desire for this form of conservation.

N. C. Ellstrand. Landraces have continually been subjected to the effects of external pollination. Much will depend on how GM-free you wish to keep the landraces. Location will also be important; it is difficult to keep landraces free in Iowa—easier in Arizona. It will further depend on a breeding system for species. Wheat may be only a limited problem.

A. G. Stephenson (*Department of Biology, Pennsylvania State University, PA, USA*). This presentation ties the two days' topics together. Inbred wild gourds are more susceptible to herbivores and pathogens, but, currently, transgenic squash are available that are resistant to several viral diseases. If (more likely, when) these virus-resistant transgenes escape into wild populations, they have the potential to dramatically alter the magnitude of inbreeding depression. This, in turn, could affect population size, especially the persistence of small populations that experience higher levels of inbreeding.

L. Comai (*Department of Biology, University of Washington, Seattle, WA, USA*). Can you identify crop species that pose no gene-flow hazard?

N. C. Ellstrand. Gene flow itself is not a hazard. It is the 'exposure' component of the risk equation. However, if you mean which major crop species are not known to spontaneously mate with wild relatives, the list is remarkably short: groundnut *Arachis hypogaea* (also known as 'peanut'), chickpea *Cicer arietinum* (also known as 'garbanzo beans') and possibly sweet potato, *Ipomoea batatas*.

L. Comai. What are the best crops for industrial chemicals?

N. C. Ellstrand. From a biosafety point of view, the worst species are outcrossing food crops that are widely planted, and that produce abundant pollen and small, easily dispersed seeds. From a biosafety point of view, the movement of genes that produce non-edible compounds would probably cause the least mischief if they were engineered into sexually sterile non-food crops that could be grown in greenhouses.