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23 Gene flow from tree plantations and implications for transgenic risk assessment

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Abstract

Gene flow is a major determinant of impacts of forest tree plantations on surrounding populations and ecosystems. Realistic predictions of gene flow are therefore essential for scientifically credible assessments of the impacts of transgenic plantations. The choice of methods for measuring gene flow will be dictated by the organism and environment under study, the availability of tools and resources, and the desired scope of inference. Methods include direct tracking of propagule movement, parentage analysis, and analysis of genetic diversity in seeds, seedlings, and pollen. Gene flow estimates can be integrated with ecological and demographic data in spatially-explicit simulation models to allow projections of transgene dispersal over large areas and long time

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frames. Such models allow exploration of a large number of scenarios of plantation cultivation, and help to identify the key parameters controlling gene flow. We describe a simulation study of gene flow from hybrid poplar plantations that illustrates some of the key issues in estimating and modeling transgene flow from plantations. For example, the results suggested that accurate estimates of long-distance gene flow are considerably more important to prediction of transgene dispersal than are estimates of local (<1 km) dispersal patterns.

Introduction

A major issue for plant genetic engineering is the extent to which transgenes will escape from cultivation and cause negative impacts in wild ecosystems [1-3]. Gene flow to wild relatives occurs for nearly all crops in some places where they are grown [4]. However, it is of particular concern for forest trees because they are virtually undomesticated [5], they have the potential for spatially extensive gene flow [6,7], and they can have large effects on ecosystem processes and biological diversity when they are the dominant life form [8].

The ecological impacts of transgenic trees will primarily depend on the traits conferred by the transgene and the environment in which the trees are grown [9,10]. Risk assessment therefore requires detailed consideration of the specific ecological consequences of individual transgenes in different settings. However, gene flow is a prerequisite for most ecological impacts outside of plantations, so baseline estimates of introgression will apply to most environmental risk assessments for transgenic trees [11,12].

Many commercially-grown trees are cultivated in close proximity to interfertile wild relatives, and many exotic plantation species have naturalized feral populations with which they can interbreed [8,13]. However, because of their inherent ecological novelty, exotic tree introductions can have large effects on ecosystems in the absence of transgenes [14]. Therefore, cultivation of exotic transgenic trees raises qualitatively different issues than cultivation of native transgenic trees. Also, because exotic trees are readily identified in wild settings, gene movement is simpler to monitor than for native species—where genetic techniques that distinguish morphologically similar progeny are required. In this chapter we will outline approaches to measuring transgene introgression, emphasizing plantations of native species. We will then discuss a risk assessment for transgenic trees, focusing on a simulated planting of transgenic hybrid poplars in the United States.

Measuring gene flow

Gene flow is a complex process that can be broken down into the following components:

- Dispersal from plantations,
- Dispersal in wild populations,
- Landscape dynamics and availability of establishment sites,
- Establishment and competitiveness of conventional plantation progeny, and
- Effects of transgenes on fertility, establishment, growth, and mortality.

Each of these components is amenable to direct measurement, and estimates can subsequently be combined using computer modeling [15-17]. We begin with a description of a variety of methods for measuring dispersal, focusing on utility for estimating gene flow from plantations (Table 1).

Table 1. Comparison of methods for estimating gene flow.

Method	Individual Parentage[a]	Exclusion Power Reqd.[b]	Sampling Effort Reqd.[c]	Correlates of Fertility Est.[d]	Cryptic Gene Flow Est.[e]	Genotype Error Bias[f]	Long Distances[g]	Biases[h]	References
Direct/Physical	N	NA	+	N	NA	+	+	N	[33]
Genotypic Exclusion	Y	+++	+++	Y	N	+++	++	R	[38]
Most Likely	Y	++	+++	Y	Y	++	+	HRF	[39,40]
Modified Most Likely	Y	+	+++	N	N	+++	+	DHR	[41]
Fractional Paternity	Y	+	+++	Y	Y	+	+	HRF	[42,43]
Mating Model	N	+	+++	Y	Y	+++	++	DHR	[44,45]
Pollen Structure	N	+	++	N	N	+	+++	ADF	[46]

[a]Parentage assigned to individuals within the population or inferred for whole groups
[b]Exclusion power required for genetic markers (determined by level of polymorphism and number of loci [47]); NA, not applicable.
[c]Relative difficulty of sampling (*e.g.*, number of adult and progeny genotypes required)
[d]Amenable to estimates of correlates of fertility such as fecundity and phenology
[e]Estimates or compensates for cryptic gene flow (i.e., gametes from outside sampled population with genotypes compatible with putative parents within the population)
[f]Degree to which estimates are sensitive to errors in genotypes (e.g., null alleles, mis-scored alleles); Note that some programs compensate for errors (e.g., Cervus [40], DNA·VIEW [48], and Micropat [49]
[g]Ability of the method to detect long-distance gene flow events with a reasonable level of sampling effort in realistic populations
[h]Biases in paternity assignment and/or gene flow estimates: N, no inherent biases; H, homozygosity favored; R, rare alleles favored; D, choice of underlying distribution(s) affects estimates; A, structure in adult populations reduces gene flow estimates; F, confounded by factors other than gene flow that affect prior mating probability (e.g., phenology, self-fertility)

Direct tracking of propagules

In this method an isolated point source is surrounded by receptors at various distances and directions. The point source may be an individual plant, or a relatively small plot. Receptors may be abiotic traps (e.g., a slide coated with vaseline or a simple seed trap [18,19]), or compatible plants (e.g., [20-22]. In the simplest case, the source is sufficiently isolated from congeneric sources to avoid confounding measurements, as is the case for an isolated, exotic plantation. However, this technique has also been applied for non-isolated sources where a unique marker is used to differentiate the source. Some markers that have been used successfully are transgenes [20,23,24], rare isoenzyme alleles [25-27], morphological markers [21,28], histochemical dyes [29], radioisotopes [30,31], and even tiny tags that identify the source plant [32]. A related approach involves

following the movement of insects or birds among flowers and plants as an indicator of pollen or seed dispersal distances e.g., [33,34].

Direct tracking has the advantages of simplicity and economy. In the case of abiotic dispersal, results of direct tracking experiments can be integrated into mechanistic models of pollen and/or seed movement that incorporate effects such as height of release, wind speed and direction, turbulence, and terminal velocity of propagules [35-37], thus allowing generalization from observations of individual trees in controlled experiments. A major advantage of this approach is it potentially allows modeling of a large number of species based solely on measurements of propagule characteristics [50,51]. As the mechanistic modeling approach continues to develop and computing power grows, it will become increasingly important for landscape modeling of gene flow.

A major disadvantage of direct tracking methods is that the source tree must be quite isolated from potentially confounding sources, especially if estimates of long-distance gene flow are required. Measuring dispersal from point sources almost always truncates the actual dispersal distances, as some propagules may travel beyond the most distant traps [52]. Also, direct tracking does not take into account secondary dispersal and pollen carryover effects in insect pollination, thus causing underestimates of dispersal distances [53]. Finally, in the case of abiotic receptors, differential pollination success, establishment, and survival are not estimated, all of which can cause substantial differences between potential gene flow (measured by propagule dispersal) and realized gene flow (measured by established seedlings) [54,55].

In the case of exotic plantations, direct tracking will usually be an adequate means of assessing spread of transgenics. However, phenotypic tracking is not possible where large, interfertile populations of wild or naturalized trees are present on the landscape (e.g., loblolly pine in the southeastern U.S.). In this case the most accurate way to estimate gene flow is parentage analysis.

Parentage analysis

Parentage analysis allows identification of specific genets that could have sired individual seeds or seedlings. The discriminating power of parentage analysis allows assessment of the cumulative effects of pollen competition, and the forces of selection in pollination, seed development, and establishment. In addition, the effects of phenological overlap and fecundity on reproductive success may be assessed in conjunction with the physical effects of distance and wind direction. Therefore this approach can provide a much more complete picture of the dynamics of dispersal than physical observations of propagule movement.

Parentage analysis has been a very active area of research in the past 15 years, and a number of different statistical methods for inferring genealogy are in development [54,56-58]. In trees, the most commonly used approach has been paternity analysis of seeds with known mothers. This is the most analytically tractable form of parentage analysis [40], though most of the methods described below can also be adapted to assess parentage of seedlings with unknown mothers.

Genotypic exclusion

In this method, adults are sequentially excluded from parentage if they could not have produced the inferred gametes. If exclusion probability is sufficiently high, only

the true parent(s) will remain. An offspring is assumed to have originated from outside sources if no compatible parents are found within the sampled population (e.g.,[38,59]). This method has the advantage of simplicity because the algorithms are relatively straightforward, and the result is potentially unambiguous.

One shortcoming of genotypic exclusion is it requires markers with very high exclusion power, which is determined by the number of loci and the level of polymorphism [47]. There are also several practical limitations to the method. First, gene flow estimates can be underestimated because of cryptic gene flow (i.e., successful immigrant gametes indistinguishable from locally produced gametes, [60,61]). If potential parents are not sampled exhaustively, some gene flow from outside the sampled population will be confounded with local parentage because of indistinguishable genotypes. Methods have been devised to estimate the extent of the problem, but these require substantial sampling in surrounding populations [54,61,62]. Another shortcoming of genotypic exclusion is that it discards offspring with ambiguous paternity and introduces a bias in paternity contribution, favoring males with rare alleles [54]. Finally, errors in genotyping resulting from null (undetectable) alleles, mutations, or scoring errors can lead to false exclusion of the true parent and inflated estimates of gene flow [63]. These problems can be quite serious for highly polymorphic markers such as microsatellites or AFLPs, and the extent of the errors should be assessed by comparing progeny to known parents [40]. Despite these shortcomings, this method has been effectively applied in several forest tree populations in recent years [64-66].

Most likely method

Lack of exclusion power of available markers led to the development of a number of maximum likelihood-based methods of assigning paternity [54]. The most straightforward of these is the 'most likely' method, in which likelihood ratios (LOD scores) are calculated for putative parent-offspring pairs or trios [39,40,67]. The most likely method is readily extensible to account for factors such as variation in allele frequencies among populations, cryptic gene flow, and genotyping errors [40,67]. However, there can be ambiguity in parentage assignment because there is no absolute cutoff for LOD scores for true fathers. One solution is to use computer simulations to derive critical values for the difference in LOD scores between putative parents for a desired level of confidence [40]. Simulations can also be used to designate LOD thresholds minimizing type I and type II errors in testing the null hypothesis of siring by local males [67].

Fractional paternity

Both the genotypic exclusion and the 'most likely' methods usually result in failure to distinguish between multiple, equally likely parents for some progeny. These progeny with ambiguous parentage are usually dropped from subsequent analyses, which introduces a bias toward detecting parentage for males with rare alleles and, for the 'most likely' method, high homozygosity [42,54]. The 'fractional paternity' method compensates for these biases by dividing parentage among all compatible individuals, proportional to their likelihood [42,54]. This allows all progeny to be included in analyses of mating success, which is especially important when a large number of

progeny have ambiguous parentage due to lack of exclusion power [43]. Fractional paternity assignment does have a tendency to bias parentage estimates toward the null hypothesis of equal fertilities, though this bias diminishes with increased exclusion power and number of offspring analyzed [42,54].

Modified most likely method

This method takes advantage of prior information on factors affecting mating success to condition likelihood estimates. For example, information on distance and direction between putative parents and seeds, fecundity, and phenological overlap can be used to calculate a prior probability of paternity [41,68]. The prior probability may be calculated by deriving an equation to describe the relationship between the aforementioned factors and mating success, and then dividing the expected value for each male by the total of the expected values for all males in the population [69]. This method makes use of data for a much larger proportion of the sampled progeny than methods based solely on genotypes [41]. A Bayesian approach can also incorporate known prior probabilities of siring into an extension of the fractional paternity framework [58]. However, if the goal of the analysis is to determine the importance of parent-offspring distance, fecundity, and phenological overlap in determining mating success (as is often the case), the reasoning is circular and prior mating functions can introduce considerable bias [42,54]. Therefore, the modified most-likely method should not be used unless the information underlying the prior probability of paternity is very reliable [54].

Mating models

This approach models the probability structure of entire samples of offspring genotypes and estimates the parameters of interest to describe mating patterns [44,70,71]. Adams and Birkes [44] originally proposed a method that simultaneously estimates the proportion of self-fertilization, and divides all outcrossing into two categories (i) local matings with genotyped males located within an arbitrarily designated neighborhood population (hence the approach is often referred to as the "Neighborhood Model"), and (ii) distant matings with individuals located outside of the neighborhood (background pollination) (see [72,73] also). Instead of determining a set of individual fertilities within neighborhoods, the neighborhood model estimates one or a few parameters (gradients) relating mating success to various selective factors such as male fecundity and flowering phenology [74,75], or ecological factors like the distance and angle between male and female parents [44,45,76].

If population-wide properties (gradients) are of interest, this approach might be superior to inferring population characteristics from previously estimated individual fertilities, especially if the accuracy of parentage assignments is limited by exclusion probabilities [75,77]. Another advantage is that the model adjusts for cryptic gene flow, and the precision of this estimate can be improved by estimating allelic frequencies of the background pollen pool [78]. Therefore, the neighborhood model provides an alternative means of investigating distant gene flow, local gene dispersal, and correlates of reproductive success simultaneously [45,76,78].

One shortcoming is that the mating model approach does not provide direct individual fertility estimates, and is therefore not amenable to assessing variation in

fertility among a large number of genotypes. Also, parentage probabilities used in the model are based on Mendelian transition probabilities, so current versions of mating models are susceptible to some of the same biases as the genotypic exclusion and fractional paternity methods described above. This approach is most appropriate for situations in which exclusion power is limited, and as corroboration for other approaches of directly estimating gene flow.

Pollen structure

A new method for estimating contemporary gene flow at a landscape level (dubbed 'TwoGener') was recently proposed by Smouse et al. [46]. This method accounts for genetic differentiation among pollen pools (Φ_{FT}) revealed through analysis of half-sib offspring samples from a number of individual mothers. Thus, unlike in paternity analysis, genotyping of potential males is not necessary. Furthermore, the Φ_{FT} parameter appears to be inversely proportional to the average distance of pollen flow, and is only moderately influenced by low exclusion power [46,79]. This parameter can be used to estimate the effective number of pollen parents (N_{ep}) and the effective pollination area (A_{ep}).

The TwoGener method shows great promise for estimating gene flow with minimal sampling and genotyping requirements compared to conventional parentage analysis. In particular, this method may provide a much-needed, efficient means of estimating long-distance gene flow in extensive tree populations. However, because the TwoGener method is quite new, it awaits rigorous assessment of the effects of some underlying assumptions. For example, the relationship between Φ_{FT} and distance relies on an assumed distribution of pollen dispersal. Although simulations indicated that TwoGener estimates differed little between exponential and normal distributions [79], it remains to be seen how the model will perform with the longer-tailed distributions more typical of tree pollen dispersal (e.g., [80,81]). Furthermore, any factors altering variation among pollen pools of different mothers, including spatial adult genetic structure, differences in female phenology, specific combining abilities, and self-fertility, if ignored, will increase Φ_{FT} estimates and bias the neighborhood size [82]. Since there have been few applications of the pollen structure approach to real populations thus far [46,83], further work is needed to investigate its properties and robustness.

Choice of markers

Regardless of the analytical technique, the accuracy of parentage analysis depends on the characteristics of the molecular markers. Parentage analysis has been revolutionized by the increasing availability of highly polymorphic markers such as microsatellites [84] and AFLPs [85] (see refs 86 and 87 for reviews). The key considerations for marker choice are:

1. *Codominance*. The ability to distinguish heterozygotes increases the exclusion power of individual loci, though probabilistic methods have been developed for use with large numbers of dominant loci such as AFLP or RAPD [88,89].
2. *Polymorphism*. Exclusion power increases with polymorphism, but care must be taken to select loci with minimal occurrence of null alleles and mutation, which tend to be high in the more polymorphic markers [90,91].

3. *Gametic equilibrium*. Most parentage methods rely on Mendelian transition probabilities, assuming genetic equilibrium among segregating alleles (i.e., no tight linkage among loci). However, increasing the number of assayed loci may confound results because substantial linkage among loci becomes more likely. This is of particular concern for methods that employ dominant, anonymous markers such as AFLPs or RAPDs [90].
4. *Cost*. Large numbers of samples must be analyzed for paternity analysis in natural populations, particularly if estimates of long-distance gene flow are required. Cost of development and/or assays generally increases with the level of polymorphism and precision of the markers [86].

Choosing a method

Clearly, the choice of methods for measuring gene flow from plantations will be dictated by the availability of markers and the species under study. If an easily assayable diagnostic marker is available, direct tracking methods are the clear choice because of ease and economy of application. However, if progeny originating from the source plantation are not readily identified, more complex parentage analysis approaches are required. Genotypic exclusion provides the clearest and simplest results, but requires extremely high-quality genetic markers with codominance, high polymorphism, and low genotyping error rates. Available markers will fall short in many cases, and likelihood-based methods are needed to compensate for marker deficiencies. With adequate exclusion power, the 'most-likely' method is an excellent choice because of its accuracy and flexibility in dealing with shortcomings such as dominance, genotyping error, and incomplete sampling. If exclusion power is low, methods such as fractional paternity and mating models provide attractive alternatives for estimating gene flow. Finally, analysis of pollen structure holds great promise for addressing the previously intractable problem of estimating long-distance gene flow in continuous populations.

Measuring habitat creation and disturbance

Rates of establishment and mortality of plantation progeny will be dictated by availability of habitat on the landscape and disturbance regimes. Standard landscape ecology techniques can be used to estimate rates of landscape change from a chronosequence of air photos or satellite data (e.g., [92]). In the context of gene flow from plantations, the key transitions will involve establishment and disappearance of trees outside of plantations. Habitat types can be defined based on the probabilities of tree establishment and prevailing successional sequences (e.g., [93,94]).

Estimating competitiveness

By competitiveness we are referring to a combination of fitness components such as establishment, growth, survival, and fecundity [16,95]. Ultimately, the only way to determine the competitive effects of a transgene in wild ecosystems is to perform field experiments in an appropriate range of environments with background genotypes sampled from populations for which transgene introgression is a concern [96-99]. Kareiva et al. [97] describe relatively simple methods for comparing invasiveness of transgenic and nontransgenic plants. To a certain degree, such assessments could be

derived from well-designed field trials, which will be undertaken in any case to assess the commercial performance of transgenic trees. However, field trials provide limited information about potential performance in wild ecosystems because the seedling stage is skipped, resources are usually abundant, and most competitors and pests are controlled. More realistic experiments are required if large-scale introgression is a genuine concern.

Spatial simulation modeling

Transgenic risk assessment requires extrapolation of spatially and temporally restricted gene flow observations to scales that are relevant for policy makers [92,100]. Because of the time and expense required for a typical gene flow study, only a limited number of populations and years can be examined [55,101]. However, ecologically significant levels of establishment may occur only once or twice per generation (i.e., on a decadal scale) [10,97,102], and in particular habitats. An emerging solution is the use of spatial simulation models [15,103], which provide an extensible framework for integrating data from disparate demographic and genetic field studies with landscape-scale analyses of ecosystem dynamics [17,56]. In addition, such models allow 'virtual experiments' through sensitivity analyses in which selected components of the system are manipulated to determine their importance in determining long-term outcomes [92,104].

Case study: Gene flow in poplar

We implemented a large-scale, multi-year effort aimed at examining potential invasiveness of transgenic poplars in the Pacific Northwest of the United States [105]. The plantations primarily consisted of first-generation hybrids between the native *Populus trichocarpa* Hooker and the introduced *Populus deltoides* Marshall. The primary objective of these studies was to provide data for assessing the extent of transgene dispersal that is likely to occur should transgenic hybrid poplars be cultivated in the region. Because of the presence of exotic and native germplasm in the plantations, and the presence of large populations of native trees, this study had characteristics of both exotic and native plantings. Therefore, we were able to track gene flow using morphological characteristics present only in plantation trees, as would be the case with truly exotic plantings, but we also addressed gene flow in native populations due to the possibility of advanced generation introgression [106]. We also gathered data on seedling establishment and survival in experimental plots and in the wild [106]. Finally, we inferred landscape-level spatial and temporal dynamics of poplar establishment from a chronosequence of GIS layers encompassing some of the same populations included in the field studies (Figure 1).

We combined all of this information in a spatial simulation model, STEVE (Simulation of Transgene Effects in a Variable Environment) (Figure 2, [105]). The STEVE model operates on a landscape grid consisting of 100 m^2 cells containing information about elevation, habitat type, and poplar populations. The simulation has an annual time step, with modules to depict creation and conversion of poplar patches, growth, reproduction, dispersal, and competition within poplar cohorts. The simulations track two genotypes: transgenic and conventional trees. Transgenic trees originate in plantations and may spread to the wild through pollen, seed, and/or vegetative

propagules, which are produced in each location proportional to basal area (i.e., trunk cross-sectional area) of each genotype, modulated by a fecundity factor.

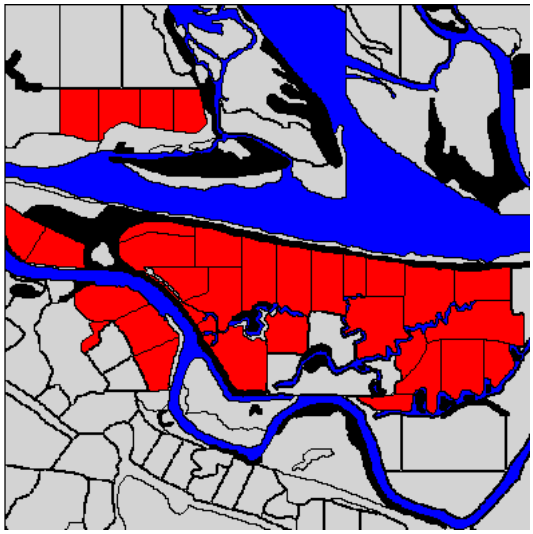

Figure 1. GIS representation of a portion of the study area in northwestern Oregon (5 km x 5 km). Red represents areas of hybrid cottonwood plantations, black, wild black cottonwood stands, gray, other cover types (mostly farms, coniferous uplands, and wetlands), and blue represents waterways, including the Columbia River [105]).

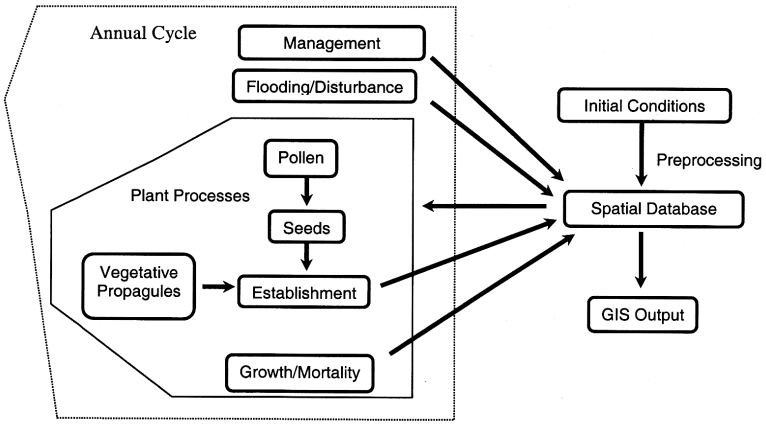

Figure 2. The STEVE model. Model begins with preprocessing of GIS layer representing initial simulation conditions. Data are stored in a spatial database containing information about elevation, cover type, poplar populations, plantations, and agricultural fields. Simulation begins with management activities such as plantation harvesting and herbicide spraying. Poplar establishment and mortality is simulated in the disturbance function. Seed, pollen, and vegetative propagules are produced proportional to basal area of each genotype, followed by dispersal, establishment, growth, and mortality. Outputs are text files and spatial data layers.

We structured and parameterized the model based on results of our field studies of gene flow. These studies used a combination of genotypic exclusion and 'most likely' methods to assign parentage within large, exhaustively sampled areas [107]. Our results indicated that long-distance dispersal is considerable for *Populus* (Table 2), with the tail of the distribution quite 'fat' [108]. We therefore chose to model gene dispersal as a two-stage process, with local dispersal modeled explicitly by a negative exponential distribution, and long-distance dispersal modeled as if a portion of the pollen and seeds were panmictic at the landscape scale. This is analogous to a mixed model approach [81,108]. The biological basis for this approach is that locally dispersed pollen grains and seed are subject to local air flows and eddies, and follow predictable patterns of dispersal in which probability of deposition declines exponentially with distance from the source (e.g., [33,35]). However, a portion of the pollen can be caught in updrafts and escape from local air flows, potentially traveling great distances (e.g., [109,110]). Seed movement was modeled in the same way, though with more attenuated dispersal curves than for pollen. *Populus* seeds are very light and contain cotton appendages that facilitate wind and water dispersal; therefore, a portion of the seeds also is expected to attain stochastic long-distance dispersal [94].

Table 2. Population and gene flow statistics from three microsatellite-based studies of pollen dispersal in Oregon, USA [107].

Site	r[a]	Mothers[b]	Fathers[c]	N[d]	D_i[e]	d_{wp}[f]	d_{op}[g]	P[h]	M[i]	G[j]
Willamette	0.25	5	221	**231**	100-300	138	**1471**	**79**	**23**	**56**
Luckiamute	1	5	57	**393**	1000-1100	128	-	**90**	**7**	**75**
Vinson	10	28	54	**681**	2680-9760	1093	**1419**	**279**	**216**	**27**

[a] Radius of sampled area (km)
[b] Number of trees from which seeds were collected
[c] Number of reproductively mature male trees within sampled area
[d] Number of progeny genotyped
[e] Distance to the nearest population of the same species (m)
[f] Average pollination distance within the reference stand (m)
[g] Mean pollination distance from assumed dispersal curve (m)
[h] Number of seeds for which a single father was compatible within the sampled area
[i] Number of seeds for which multiple fathers were compatible
[j] Percentage of seeds for which no compatible fathers were identified

This method of modeling pollen and seed dispersal had major implications for gene flow from transgenic plantations. Modeled gene flow was relatively insensitive to the slope of local dispersal curves (Figures 3A and B), but highly sensitive to changes in the proportion of pollen and seed dispersed long-distances (Figures 3C and D). This was primarily because poplars require very intense disturbance, abundant moisture, and freedom from most competition by other plants for successful establishment. These conditions are rarely met in space and time. The majority of establishment sites therefore occurred primarily beyond the local seed and pollen shadows of the plantations (Figure 3E). Also, because long-distance dispersal was insensitive to wind in this model (pollen and seed were assumed to be panmictic at the landscape scale), wind speed had

no detectable effect on gene flow from plantations (Figure 3F). Long-distance dispersal ensured that a proportion of plantation-derived propagules would encounter stochastic establishment sites regardless of distance from plantations, which explains why this portion of the dispersal function was overwhelmingly important in determining gene flow. One implication of this result is that future research on gene flow in *Populus* would benefit most from better definition of the dynamics of long-distance dispersal,

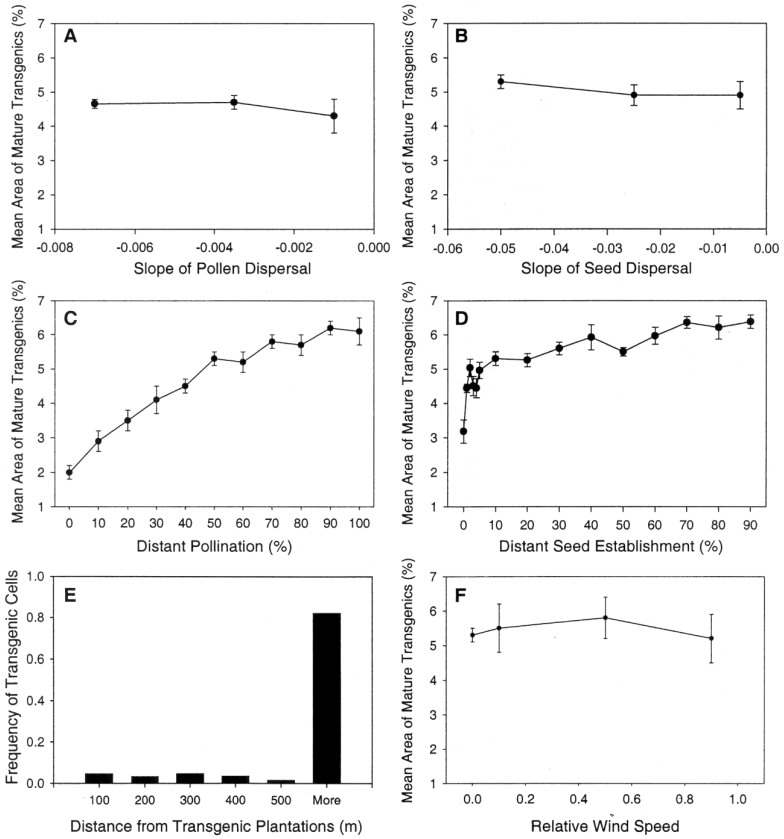

Figure 3. Effects of dispersal and wind on simulated gene flow (the percent area of mature wild poplar stands occupied by transgenic trees after 50 years of simulation). Error bars are 1 standard error from 10 repetitions with each set of parameter values. **A and B**. Effects of varying the slope of the negative exponential distributions depicting local pollen, and seed dispersal, respectively. Varying this slope had little effect on gene flow. **C.** Effects of distant pollination on transgene flow. Distant pollination is the proportion of seeds that are fathered by trees that do not occur in the local population. This parameter has a strong effect on transgene flow, reflecting the importance of long distance pollen dispersal. **D.** Effects of distant seed establishment on transgene flow. Distant seed establishment had relatively minor effects except at very low levels. **E.** Distance of transgenic cohorts from mature transgenic plantations. The local pollen and seed shadows end at 440 m and 220 m respectively. **F.** Effect of relative wind speed, with wind direction set at 90 degrees.

rather than from studies of local pollen movement and mating between trees within stands. This is consistent with findings from many other studies of invasion processes [101,108,111]

Sensitivity analyses allowed us to study the consequences for gene flow of many ecological conditions and transgenic deployment scenarios over a 50 to 100 year time frame [105]. For example, we studied the consequences of:

1. Transgenes that imparted herbicide resistance with respect to various scenarios of herbicide use and disturbance on the landscape
2. Transgenic trees with insect resistance, with varying levels of insect attack in wild populations
3. Reduced fertility of plantations, and implications of various levels of efficiency and stability of transgenic flowering control
4. Effects of transgenic plantation area, gender, and rotation length.

Most of these simulations also included stochastic variation, so that natural environmental variances and uncertainty in parameter estimates could be reflected in model outputs. Ideally, the model structure and parameters would be continually revised based on research results, and by results of monitoring programs during commercial deployment.

The most important contribution of spatial simulation models is that they provide a comprehensive, explicit, and logical framework for thinking about the long-term consequences of different options for deploying transgenic and conventional trees. Modeling helps to reduce the immense ecological complexity of tree gene flow to a set of specific, testable predictions to guide further research. Models like STEVE can therefore play an important role in ensuring safe and responsible deployment of transgenic technology in plantation forestry.

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