Using empirical data to model transgene dispersal

T. R. Meagher^{1*}, F. C. Belanger² and P. R. Day³

¹*School of Biology, Division of Environmental and Evolutionary Biology, Sir Harold Mitchell Building, University of St Andrews, St Andrews KY16 9TH, UK*

²*Plant Biology and Pathology Department, and* ³*Biotechnology Center for Agriculture and the Environment, Rutgers University, New Brunswick, NJ 08901-8520, USA*

One element of the current public debate about genetically modified crops is that gene flow from transgenic cultivars into surrounding weed populations will lead to more problematic weeds, particularly for traits such as herbicide resistance. Evolutionary biologists can inform this debate by providing accurate estimates of gene flow potential and subsequent ecological performance of resulting hybrids. We develop a model for gene flow incorporating exponential distance and directional effects to be applied to wind-pollinated species. This model is applied to previously published data on gene flow in experimental plots of *Agrostis stolonifera* L. (creeping bentgrass), which assessed gene flow from transgenic plants resistant to the herbicide glufosinate to surrounding non-transgenic plants. Our results show that although pollen dispersal can be limited in some sites, it may be extensive in others, depending on local conditions such as exposure to wind. Thus, hybridization under field conditions is likely to occur. Given the nature of the herbicide resistance trait, we regard this trait as unlikely to persist in the absence of herbicide, and suggest that the ecological consequences of such gene flow are likely to be minimal.

Keywords: transgenic herbicide resistance; gene flow; paternity analysis; *Agrostis palustris*; turfgrass; risk assessment

1. INTRODUCTION

The debate surrounding the use of genetically engineered crops in the UK and elsewhere has underscored the need for an understanding of the potential for spread of transgenes beyond cultivation. Indeed, there has been considerable concern that transgenic effects such as herbicide resistance will spread beyond the target crop species, resulting in novel weed biotypes that will be difficult to control. Two key elements for which scientific methods can inform this debate are the potential rate of gene flow into non-crop species and the fitness consequences of such gene flow should it occur.

Gene flow can be broken down into two components: gene dispersal and cross-fertility. Because the focus of discussion on transgene dispersal is often pollen dispersal and subsequent hybridization with weedy relatives of crop species (Dale 1994; Darmency 1996; Ellstrand *et al.* 1999; Johnson & Riordan 1999), either or both components of gene flow can be usefully investigated by analysis of pollen donor fitness variation. The application of genetic-marker-based methods of paternity analysis to natural plant populations has provided new insights into pollen donor (male) fitness variation (Meagher 1986, 1991; Roeder *et al.* 1989; Adams & Birkes 1991; Smouse & Meagher 1994), and the models of fitness variation resulting from such approaches (Sork *et al.* 1998, 1999; Smouse *et al.* 1999; Morgan &

Conner 2001; Meagher & Vassiliadis 2003) provide an effective tool for assessing potential gene flow patterns in transgenic crop systems.

The present paper develops and applies spatial models of pollen fitness variation to determine pollen dispersal patterns. The models incorporate both distance and direction, two spatial dimensions likely to be relevant to many crop species for which pollen is wind dispersed. These models will then be applied to data on transgenic pollen dispersal from experimental plots of the turfgrass *Agrostis stolonifera* L. (creeping bentgrass).

2. MODELLING POLLEN DISPERSAL

Pollen-donor fitness variation can be assessed in genetic-marker-based paternity analysis by estimation of an ML distribution of pollen donor fertilities in which the overall gene pool in the progeny generation is allocated proportionately to the candidate pollen donors (Roeder *et al.* 1989; Smouse & Meagher 1994). The outcome of such analysis is a distribution of pollen donor fertilities, $\{\lambda_k\}$, over the $k = 1, 2, \dots, K$ candidate pollen donors where $\sum \lambda_k = 1$. Studies of pollen-donor fertility distributions in natural populations have shown that there is considerable variation in pollen donor success, with many plants contributing little and a few plants dominating the offspring gene pool (Meagher & Vassiliadis 2003). For example, in a paternity analysis of the dioecious species *Chamaelirium luteum* (Meagher 1986; Smouse & Meagher 1994), the ML distribution of $\{\lambda_k\}$ was found to be significantly non-uniform, with as many as 55 out of 273 candidate pollen donors making essentially no genetic contribution (figure

*Author for correspondence (trm3@st-and.ac.uk).

One contribution of 21 to a Discussion Meeting Issue 'Mechanisms regulating gene flow in flowering plants'.

Figure 1. Individual male fertilities $\{\lambda_k\}$ of individuals in a population of *Chamaelirium luteum* estimated by ML paternity analysis.

1). A major goal of paternity analysis is to determine the underlying causality of such variation.

One contributing factor to pollen success is the proximity of the pollen source to potential pollen recipients (Meagher & Vassiliadis 2003). Investigation of this factor amounts to evaluating pollen dispersal. In plant populations, a widely used model of pollen dispersal is a negative exponential function in which pollen availability experiences an exponential decay with increasing distance from the source. ML studies of pollen donor fertility incorporating such distance effects have shown that exponential decay of pollen dispersal explains much of the variation in pollen donor fertility. Impacts of distance are quantified by using an inter-mate distance function:

$$\delta_{ik} = \ln \{ [x_{\mathbf{q}_i} - x_{\mathbf{q}_k}]^2 + [y_{\mathbf{q}_i} - y_{\mathbf{q}_k}]^2 \}^{0.5} \tag{2.1}$$

resulting in the model

$$\ln \lambda_{ik} = \gamma \delta_{ik} \tag{2.2}$$

which implies the following estimator for the fertility of the k th pollen source on the i th pollen recipient

$$\lambda_{ik} = e^{\gamma \delta_{ik}} / \sum e^{\gamma \delta_{ik}}. \tag{2.3}$$

Under this model, pollen dispersal from source plants shows an exponential dispersal according to the value of parameter γ , where γ is typically expected to be negative so that pollen dispersal shows an exponential decay (figure 2).

Given the potential importance of direction as well as distance, a working model of pollen dispersal should include a directional component. The exponential model of pollen dispersal was expanded by Burczyk *et al.* (1996) to include such a directional component. Thus, a more complete model of spatial effects on pollen dispersal is given by

$$\ln \lambda_{ik} = \gamma \delta_{ik} + \alpha \phi_{ik} \tag{2.4}$$

which, in turn, implies the following estimator for the fertility of the k th candidate pollen donor on the i th pollen recipient

$$\lambda_{ik} = e^{(\gamma \delta_{ik} + \alpha \phi_{ik})} / \sum e^{(\gamma \delta_{ik} + \alpha \phi_{ik})}. \tag{2.5}$$

This models pollen dispersal in terms of both distance

Figure 2. Expected relative male reproductive success $\{\lambda_k\}$ according to a negative exponential model of pollen dispersal (equation (2.2)) with different values for the distance effect parameter γ .

(δ_{ik}) and angular direction (ϕ_{ik}) between the k th candidate pollen donor and the i th pollen recipient, where angular direction is defined as the absolute value of the departure from the average directional angle of mating events that have been observed. Measured in radians, ϕ_{ik} ranges in value from 0 to π .

3. TRANSGENE DISPERSAL IN EXPERIMENTAL PLOTS OF AGROSTIS STOLONIFERA

The preceding model was applied to data on observed dispersal of transgenes in the turfgrass *A. stolonifera*. A major management issue in golf greens, where this turfgrass species is widely used, is control of other grass species, such as *Poa annua* L. Given that such weeds have very similar herbicide tolerances to *A. stolonifera*, essentially, they cannot be controlled once they have become established. Thus, there is potential for considerable improvement in greens' management through the introduction of herbicide-resistant varieties of *A. stolonifera*. Transgenic biotypes of *A. stolonifera* with resistance to the herbicide glufosinate were generated in the Biotech Center of Rutgers University (Hartman *et al.* 1994; Lee *et al.* 1996), and these biotypes provided material for a subsequent investigation of transgene flow under field conditions (Belanger *et al.* 2003).

Table 1. Recovery of intraspecific (*A. palustris*) transgenic hybrids (from Belanger *et al.* 2003).

	1999		2000	
	transgenics	total screened	transgenics	total screened
plot 1	40	70 845	410	67 255
plot 2	133	55 084	573	88 518
total	173	125 929	983	165 773
%		0.317		0.593

To evaluate gene flow, Belanger *et al.* (2003) established two experimental plots at the Rutgers Agricultural Research Center in Upper Deerfield, NJ, in the summer of 1999. Each plot consisted of a hexagonal grid with five transgenic pollen source plants at the centre of the plot and five pollen recipient plants and 90 grid points (each containing one plant of *A. canina* L., *A. capillaris* L., *A. stolonifera*, *A. gigantea* Roth., and *A. castellana* Boiss. and Rent.). Each grid point was separated from adjacent points by 3 m, resulting in a maximum transgene dispersal distance within a plot of 15 m. The two plots were ca. 140 m apart. All pollen recipients were non-transgenic, and preliminary screenings were done to ensure that there was no naturally occurring resistance to the herbicide glufosinate among them. Seed were harvested from pollen recipient plants in the summers of 1999 and 2000 and screened for resistance to glufosinate. The objectives of this study were to assess transgene pollen dispersal distances and the potential for interspecific transgene hybridization under field conditions. In summary, there were transgenic progeny recovered from intraspecific pollen recipients, but considerably fewer recovered from interspecific pollen recipients, with some of the other species showing no herbicide resistance in their progeny. The intraspecific recovery of transgenes among pollen recipients, although at a low frequency, was still high enough to provide a reasonable empirical basis for evaluating models of pollen dispersal (table 1; figure 3); and a directional component of intraspecific transgene dispersal, presumably reflecting prevailing winds, was also noted. The present analysis is based solely on intraspecific gene flow in these plots and will provide a formal model of transgene pollen dispersal.

To fit the *Agrostis stolonifera* data to the spatial model for distance and directional effects on pollen dispersal (equation (2.5)), a nonlinear regression was performed using PROC NLIN in SAS (SAS Institute 2001) to estimate γ and ϕ for each plot and year combination using a regression model based on equation (2.5). Regressions were based on a unitary distance between adjacent points on the hexagonal grid such that 1 unit equals 3 m. All four regressions yielded a significantly good fit (table 2) and were subsequently used to generate expected transgenic pollen dispersal to each point on the hexagonal grid (figure 4).

These analyses show that the expected dispersal of pollen in the first year was limited, but increased considerably in the second year, with a strong directional component in both years. Plot 1, with a strong negative distance effect in 1999, provides a good illustration of a negative exponential decay in pollen dispersal. The realized disper-

sal of pollen was over a greater distance in the second year, because the quantity of pollen being dispersed was greater. This is reflected in the negative but weak distance effect parameter estimates for both plots in 2000. The fact that plot 2 showed a similarly weak negative distance effect in both years, reflecting potential for considerable gene flow beyond the limits of the plot, is probably a location effect reflecting greater exposure to wind. In conclusion, pollen dispersal profiles seem to be consistent over different years, though the amount of pollen available for dispersal can vary considerably; and site and year are very important, with some showing limited pollen dispersal and others showing extensive dispersal.

4. DISCUSSION AND CONCLUSION

The results of this study show that the pollen dispersal from point sources can be extensive. Therefore, in the case of wind-pollinated cultivars, opportunities for hybridization will occur. Indeed, there are recent reports of transgene flow into weed populations by hybridization (e.g. Hall *et al.* 2000). Our present results suggest that risk assessment analyses based primarily on gene flow by pollen transfer may be misdirected. One of the predictions from population genetics theory is that even very low levels of gene flow are sufficient for the spread of a trait if it is favoured by selection. However, a trait that is not favoured by selection is unlikely to be sustained even if there is gene flow. Thus, the focus of attention in risk assessment should be on the relative fitness of plants that have acquired the transgene, and the possibility of transgenic traits being favoured by selection will in turn be very much dependent on the trait in question.

In the case of herbicide tolerance, there is reason to expect that transgenic herbicide tolerance would not persist in natural habitats, or that if it did escape it would be more a management issue than an ecological concern. Two lines of reasoning, and potential experimental risk assessment, are worth pursuing further. The first is to evaluate the fitness of either the transgenic cultivar or biotypes of related weed species to which the transgenic herbicide resistance has been transferred by hybridization. The second is to consider the relative rates of acquisition of herbicide resistance through transgenic hybridization versus *de novo* evolution of herbicide resistance.

If there are fitness costs associated with a trait, removal of selection favouring a trait will cause such traits to degenerate or disappear. In the case of herbicide resistance, the selection favouring its maintenance is artificial, based on application of the appropriate herbicide. Another factor in considering fitness consequences is the overall

Figure 3. Observed counts of transgenic progeny over the hexagonal grid for each plot–year combination: (a) plot 1, 1999; (b) plot 2, 1999; (c) plot 1, 2000; (d) plot 2, 2000.

Table 2. Nonlinear regression coefficients estimated for each plot–year combination using the model $\ln \lambda_{ik} = \gamma \delta_{ik} + \alpha \phi_{ik}$.

	1999			2000		
	γ	α	p -level	γ	α	p -level
plot 1	−0.29	−0.87	< 0.0001	−0.03	−1.29	0.0007
plot 2	−0.06	−0.99	0.0003	−0.06	−0.37	0.0002

environmental background. For example, Snow *et al.* (1999) reported that transgenic herbicide resistance in hybrids between *Brassica napus* and *B. rapa* showed no apparent impact on fitness in controlled environment growth chambers, whereas evaluation of *B. napus* under field conditions (Crawley *et al.* 1993) showed a reduction in fitness in transgenic herbicide resistant biotypes. The relatively few studies of fitness consequences that have been done so far under field conditions suggest that transgenic herbicide tolerance will reduce fitness in comparison with plants that do not express that trait (see Crawley *et al.* 1993, 2001; Hails *et al.* 1997).

Another consideration is that herbicide usage represents a very strong short-term selection agent, and there is a general trend that herbicide resistance in weed populations usually develops within the first few years of use of a new herbicide (Gould 1991). In the UK, there are some 23 weed biotypes that express herbicide resistance, and as many as 272 worldwide (Heap 2003). Very little is known about the relative fitness of naturally occurring herbicide resistant biotypes; but for the only resistant category in the UK for which information is available, triazine

resistance, reduced fitness has been documented under field conditions (Jordan 1999). Thus, herbicide resistance in weeds is a likely consequence of herbicide usage, whether or not transgenic plants are involved. A plant breeding strategy that includes transgenic herbicide tolerance does not change the basic rules of the arms race between crops and problematic weeds, but it does provide plant breeders with a useful tool for staying a step or two ahead of weeds.

One of the most successful applications of transgenic herbicide resistance has been the introduction of Roundup Ready varieties of crops such as corn and soybeans. Roundup (glyphosate) has a low environmental impact and has been in use for nearly 30 years with relatively little emergence of resistance in weeds (Bradshaw *et al.* 1997). However, glyphosate resistance has been appearing in recent years, presumably in response to intensive use of glyphosate in conjunction with glyphosate-resistant crops (see Paoletti & Pimentel 2000; Pollack 2003). Current opinion is that this represents more of a management problem than an ecological one. Appropriate management strategies could slow down or prevent the spread of

Figure 4. Predicted counts of transgenic progeny over the hexagonal grid (see table 2 for nonlinear regression coefficients) for each plot–year combination: (a) plot 1, 1999; (b) plot 2, 1999; (c) plot 1, 2000; (d) plot 2, 2000.

herbicide resistance in weed populations by removing the selection agent that promotes it (Gould 1995; Jasieniuk *et al.* 1996; Christoffers 1999; Friesen *et al.* 2000).

In summary, it is useful to know that transgene transfer by hybridization can take place under field conditions. Moreover, it is useful to know that transgene transfer between species occurs at a relatively low level. However, that is in itself not a sufficient information base for a risk assessment, which should include evaluation of fitness consequences under field conditions. Even so, the scientific and practical experience base so far suggests that in the case of herbicide resistance, genetic engineering *per se* is considerably less likely to lead to problems than mismanagement of application.

The authors thank Polina Kogan, Cindy Laramore, Jessica Parezo, Harry Hodge and Kenneth Shallop for excellent assistance, William Meyer for consultation on turfgrass, and Christine Vassiliadis for comments on the paper. This work was supported by grant 97-39210-5008 from the United States Department of Agriculture.

REFERENCES

- Adams, W. T. & Birkes, D. S. 1991 Estimating mating patterns in forest tree populations. In *Biochemical markers in the population genetics of forest trees* (ed. S. Fineschi, M. E. Malvolte, F. Cannata & H. H. Hattemer), pp. 157–172. The Hague: SBP Academic.
- Belanger, F. B., Plumley, K., Meyer, M., Day, P. R. & Meagher, T. R. 2003 Interspecific hybridization between *Agrostis palustris* (creeping bentgrass) and related *Agrostis* species under field conditions. *Crop Sci.* **43**, 240–246.
- Bradshaw, L. D., Padgett, S. R., Kimball, S. L. & Wells, B. H. 1997 Perspectives on glyphosate resistance. *Weed Technol.* **11**, 189–198.
- Burczyk, J., Adams, W. T. & Shimizu, J. Y. 1996 Mating patterns and pollen dispersal in a natural knobcone pine (*Pinus attenuata* Lemmon) stand. *Heredity* **77**, 251–260.
- Christoffers, M. J. 1999 Genetic aspects of herbicide-resistant weed management. *Weed Technol.* **13**, 647–652.
- Crawley, M. J., Hails, R. S., Rees, M., Kohn, D. & Buxton, J. 1993 Ecology of transgenic oilseed rape in natural habitats. *Nature* **363**, 620–623.
- Crawley, M. J., Brown, S. L., Hails, R. S., Kohn, D. D. & Rees, M. 2001 Biotechnology—transgenic crops in natural habitats. *Nature* **409**, 682–683.
- Dale, P. J. 1994 The impact of hybrids between genetically-modified crop plants and their related species—general considerations. *Mol. Ecol.* **3**, 31–36.
- Darmency, H. 1996 Movement of resistance genes among plants. *Mol. Genet. Evol. Pest. Resist.* **645**, 209–220.
- Ellstrand, N. C., Prentice, H. C. & Hancock, J. F. 1999 Gene flow and introgression from domesticated plants into their wild relatives. *A. Rev. Ecol. Syst.* **30**, 539–563.
- Friesen, L. J. S., Ferguson, G. M. & Hall, J. C. 2000 Management strategies for attenuating herbicide resistance: untoward consequences of their promotion. *Crop Protect.* **19**, 891–895.
- Gould, F. 1991 The evolutionary potential of crop pests. *Am. Sci.* **79**, 496–507.
- Gould, F. 1995 Comparisons between resistance management strategies for insects and weeds. *Weed Technol.* **9**, 830–839.
- Hails, R. S., Rees, M., Kohn, D. D. & Crawley, M. J. 1997 Burial and seed survival in *Brassica napus* subsp. *oleifera* and *Sinapis arvensis* including a comparison of transgenic and non-transgenic lines of the crop. *Proc. R. Soc. Lond. B* **264**, 1–7. (DOI 10.1098/rspb.1997.0001.)
- Hall, L., Topinka, K., Huffman, J., Davis, L. & Good, A. 2000 Pollen flow between herbicide-resistant *Brassica napus* is the cause of multiple-resistant *B. napus* volunteers. *Weed Sci.* **48**, 688–694.

- Hartman, C. L., Lee, L., Day, P. R. & Turner, N. E. 1994 Herbicide-resistant turfgrass (*Agrostis palustris* Huds.) by biolistic transformation. *BioTechnology* **12**, 919–923.
- Heap, I. 2003 The International Survey of Herbicide Resistant Weeds. Online: www.weedscience.com, 19 January 2003.
- Jasieniuk, M., BruleBabel, A. L. & Morrison, I. N. 1996 The evolution and genetics of herbicide resistance in weeds. *Weed Sci.* **44**, 176–193.
- Johnson, P. G. & Riordan, T. P. 1999 A review of issues pertaining to transgenic turfgrasses. *Hortscience* **34**, 594–598.
- Jordan, N. 1999 Fitness effects of the triazine resistance mutation in *Amaranthus hybridus*: relative fitness in maize and soyabean crops. *Weed Res.* **39**, 493–505.
- Lee, L., Laramore, C. L., Day, P. R. & Turner, N. E. 1996 Transformation and regeneration of creeping bentgrass (*Agrostis palustris* Huds) protoplasts. *Crop Sci.* **36**, 401–406.
- Meagher, T. R. 1986 Analysis of paternity within a natural population of *Chamaelirium luteum*. 1. Identification of most-likely male parents. *Am. Nat.* **128**, 199–215.
- Meagher, T. R. 1991 Analysis of paternity within a natural population of *Chamaelirium luteum*. 2. Patterns of male reproductive success. *Am. Nat.* **137**, 738–752.
- Meagher, T. R. & Vassiliadis, C. 2003 Spatial geometry determines gene flow in plant populations. In *Genes in the environment* (ed. R. S. Hails, J. E. Beringer & H. C. J. Godfray), pp. 76–90. Oxford: Blackwell Science Ltd.
- Morgan, M. T. & Conner, J. K. 2001 Using genetic markers to directly estimate male selection gradients. *Evolution* **55**, 272–281.
- Paoletti, M. G. & Pimentel, D. 2000 Environmental risks of pesticides versus genetic engineering for agricultural pest control. *J. Agric. Environ. Ethics* **12**, 279–303.
- Pollack, A. 2003 Widely used crop herbicide is losing weed resistance. *The New York Times*, 14 January 2003.
- Roeder, K., Devlin, B. & Lindsay, B. G. 1989 Application of maximum-likelihood methods to population genetic data for the estimation of Individual fertilities. *Biometrics* **45**, 363–379.
- SAS Institute Inc 2001 *The SAS® System for Windows™, version 8.2*. Cary, NC: SAS Institute.
- Smouse, P. E. & Meagher, T. R. 1994 Genetic analysis of male reproductive contributions in *Chamaelirium luteum* (L.) Gray (Liliaceae). *Genetics* **136**, 313–322.
- Smouse, P. E., Meagher, T. R. & Kobak, C. J. 1999 Parentage analysis in *Chamaelirium luteum* (L.) Gray (Liliaceae): why do some males have higher reproductive contributions? *J. Evol. Biol.* **12**, 1069–1077.
- Snow, A. A., Andersen, B. & Jorgensen, R. B. 1999 Costs of transgenic herbicide resistance introgressed from *Brassica napus* into weedy *B. rapa*. *Mol. Ecol.* **8**, 605–615.
- Sork, V. L., Campbell, D. R., Dyer, R., Fernandez, J. F., Nason, J., Petit, R., Smouse, P. E. & Steinberg, E. 1998 Proceedings from a workshop on gene flow in fragmented, managed, and continuous populations. Research paper no. 3. National Center for Ecological Analysis and Synthesis, University of California, Santa Barbara. Online: <http://www.nceas.ucsb.edu/nceas-web/projects/2057/nceas-paper3/>
- Sork, V. L., Nason, J., Campbell, D. R. & Fernandez, J. F. 1999 Landscape approaches to historical and contemporary gene flow in plants. *Trends Ecol. Evol.* **14**, 219–224.

GLOSSARY

ML: maximum likelihood