Theor Appl Genet (1996) 93:1319–1326

C. Lavigne · B. Godelle · X. Reboud · P. H. Gouyon

A method to determine the mean pollen dispersal of individual plants growing within a large pollen source

Received: 17 December 1995 / Accepted: 26 January 1996

Abstract Pollen dispersal has been recently focused on as a major issue in the risk assessment of transgenic crop plants. The shape of the pollen dispersal of individual plants is hard to determine since a very large number of plants must be monitored in order to track rare long-distance dispersal events. Conversely, studies using large plots as a pollen source provide a pollen distribution that depends on the shape of the source plot. We report here on a method based on the use of Fourier transforms by which the pollen dispersal of a single, average individual can be obtained from data using large plots as pollen sources, thus allowing the estimation of the probability of long-distance dispersal for single plants. This method is subsequently implemented on simulated data to test its susceptibility to random noise and edge effects. Its conditions of application and value for use in ecological studies, in particular risk assessment of the deliberate release of transgenic plants, are discussed.

Key words Pollen dispersal · Gene flow · Computer simulations · Fourier transforms · Deconvolution

Introduction

Pollen dispersal has been investigated intensively by paleobotanists to reconstruct former plant communities (e.g. Tauber 1965; Faegri and Iversen 1975; Calcote 1995), by plant breeders to determine isolation distances for seed production (e.g. Bateman 1947; Griffiths 1950; Wagner and Allard 1991; Stewart 1994) and by plant population biologists to understand the genetic structure of plant populations (e.g. Levin and Kerster 1974; Schaal 1980; Bos et al. 1986; Campbell 1991), the efficiency of different pollen vectors (e.g. Honig et al. 1992) and the evolution of plant reproductive traits (e.g. Thomson and Thomson 1989; Galen 1992). Pollen dispersal from crop plants has only recently been the subject of a more specific scrutiny (see e.g. Manasse 1992; Scheffler et al. 1993; Skogsmyr 1994; Morris et al. 1994) since it is feared that transgenes might escape from transgenic crops to wild populations through hybridization and thus create new weeds (Gregorius and Steiner 1993; Crawley et al. 1993). Pollen dispersal experiments have already been performed on a few transgenic crops, such as potato (Tynan et al. 1990; McPartlan and Dale 1994; Skogsmyr 1994), oilseed rape (Scheffler et al. 1993), cotton (Umbeck et al. 1991) and sugar beet (BRIDGE 1994).

In view of the fast development of transgenic crops and the proximity of their culture on a large scale for commercial purposes, our knowledge of pollen dispersal and its consequences in terms of gene flow is, however, insufficient. A major novelty is that, contrary to most populations of wild species, transgenic crop fields emit a very large and simultaneous amount of genetically identical pollen grains. Moreover, studies with crop plants have, until recently, focused on the pollution of field crops by wild relatives but not the opposite (but see Luby and McNicol 1995). Finally, as pointed out by Tiedje et al. (1989), past experiences with disease epidemics, the release of biological control agents and the invasions of higher organisms suggest that the scale and frequency of introduction highly determine the ability of a new organism to establish so that both biologists and regulators agree that small-scale releases do not provide sufficient insight to predict the outcome of large-scale commercial releases (Stone 1994; Morris et al. 1994).

Modelling offers a potential solution by which to investigate the possible consequences of a change in the scale of releases. One of the components needed in such

Communicated by P.M.A. Tigerstedt

C. Lavigne (✉)
Institut für Umweltwissenschaften, Universität Zürich/Irchel, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland

B. Godelle · P.H. Gouyon
Laboratoire d'Evolution et Systématique des Végétaux, Université Paris-Sud XI, bât. 362, URA 1492, F-91405 Orsay Cedex, France

X. Reboud
INRA, Laboratoire de Malherbologie, BV 1540, F-21034 Dijon Cedex, France

models is the pollen dispersal of each individual plant since this allows us to test for the consequences of changing the distribution of a given transgenic crop on a regional scale. In such models, low-frequency long-distance dispersal might prove to be highly relevant for risk assessment studies since a very small number of successful migrants per generation may be sufficient to modify the genetic composition of a population (Wright 1931). Moreover, there could be important ecological impacts when the transgene is highly undesirable or provides a high fitness.

Pollen distributions which are available for such models have two distinct origins: (1) models which consider wind-dispersed pollen grains as any physical particle subject to mechanical forces and (2) pollen-dispersal experiments. The former describe wind-dispersed pollen (Okubo and Levin 1989) but do not take into account the pollen's viability or the presence of receptive individuals, features of obvious importance to risk assessment studies in which estimation of the dispersal of a transgene is desired. Experiments on pollen dispersal have used such varying means as microtags (Nilsson et al. 1992), radioactive tracers (Schlising and Turpin 1971; Massaux et al. 1976), fluorescent dyes (Waser and Price 1982; Linhart et al. 1987) and marker genes (Ellstrand et al. 1989; Galen 1992). Distributions obtained by means of these protocols usually take into account the distributions of the receptive plants but may face the problem of pollen viability when no genetic marker is used to assess the actual transfer of genetic material. Pollen distribution patterns are commonly described as being leptokurtic, negative exponential or Weibull with most of the pollen fertilising plants growing in close proximity of the source (Levin and Kerster 1974; Tonsor 1985; Morris et al. 1994). Further, most of the pollen-dispersal experiments face the problem that it is necessary to have a large source of pollen in order to track rare events of long-distance dispersal. As a consequence, the shape of the pollen dispersal obtained is that of a great number of source plants. It will therefore reflect not only the pollen distribution from each plant but also the shape of the experimental design.

The aim of this paper is to present a method which solves the apparent paradox of assessing the pollen dispersal of a unique plant whilst still taking rare long-distance dispersal events into account. This was achieved by assessing the pollen dispersal of each plant from the global pollen dispersal of a source composed of a high number of donor plants. The validity of this method, thereafter named the deconvolution method, was tested on computer data simulating a pollen-dispersal experiment with transgenic oilseed rape.

Materials and methods

The deconvolution method

Assumptions

We assume that:
1) all plants have the same mean pollen-dispersal distribution, which needs not be isotropic;
2) all-male-fertile plants produce the same amount of pollen;
3) pollen is not limiting;
4) some plants (named source plants) are homozygous for a dominant marker gene and their pollen can therefore be distinguished from that of the other plants;
5) all pollen grains are equally competitive irrespective of whether they carry the marker gene or not;
6) plant flowering is synchronous.

The total pollen received by any plant is the sum of the pollen grains it receives from every plant in the field. The proportion of marked pollen grains collected by a plant i of coordinates (x, y) can therefore be written as

$$(g^*f)(x,y) = \int_{-\infty}^{+\infty}\int_{-\infty}^{+\infty} f(x,y,x',y')g(x',y')\,dx'\,dy'$$

where $f(x, y, x', y')$ is the quantity of pollen grains from plant j (coordinates x', y') falling on plant i and $g(x', y')$ is the proportion of marked pollen among the pollen produced by plant j. $f(x, y, x', y')$ depends both on the distance between plant i and plant j and on the distribution of the distances traveled by pollen grains, which is supposedly independent of the plants considered. This distribution is also the dispersal curve of each plant in the field, assuming that all plants contribute similarly to the pollen cloud. The global dispersal function $g*f$ is the convolution of functions f and g.

Since the source plants are homozygous for the marker gene and all the other plants are recessive homozygotes at that locus, $g(x, y)$ only takes two values: 1 if j is a source plant and 0 if otherwise.

Fourier transforms

The method presented here to find the function f from the global dispersal function $f*g$ relies on the use of Fourier transforms.

Fourier transforms have been developed largely for the analysis of spectrum data. They are linear operations that provide the representation of a physical process in the frequency domain, that is, they specify it by giving amplitude as a function of frequency. In the present study, Fourier transforms were used as an efficient tool through the deconvolution theorem. The Fourier transforms and their inverse were implemented on arrays of data using the Fast Fourier Transform procedure suggested by Press et al. (1992)

The deconvolution theorem

The Fourier transform of the convolution of two functions, $g*f$, is the product of their Fourier transforms, i.e. $F(g*f) = F(g)F(f)$. Knowing $f*g$, the global pollen dispersal and g, the proportion of marked pollen produced by each plant, we can therefore derive f, from the relation

$$f = F^{-1}\left(\frac{F(g*f)}{F(g)}\right).$$

Test of the deconvolution method on simulated data

When implemented on deterministic equations, the deconvolution method clearly provides the expected result. Empirical data, however, do not necessarily meet all of the assumptions outlined earlier. It is especially unlikely that all plants have the same individual dispersal pattern and that they all contribute equally to the pollen cloud. The deconvolution method was therefore tested on an array of simulated data allowing some random effects in these two processes. The simulated field was not infinite, so that, as in field experiments, some edge effects were expected. This enabled us to test the susceptibility of the method to random noise and the possible existence of a systematic bias due to edge effects.

Simulated pollen dispersal

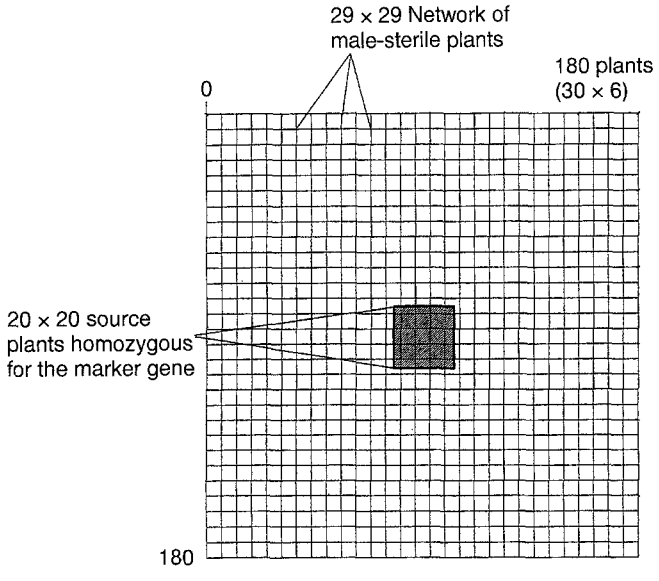
The pollen dispersal experiment was simulated on a 180 × 180 grid which would represent a field of approximately 1 ha for oilseed rape. This is the size of the large pollen-dispersal experiments that were performed by European teams with transgenic oilseed rape (e.g. Scheffler et al. 1993; Bridge 1994) and was acceptable in terms of computing time. Each node of the grid was occupied by a plant defined by its genotype at a nuclear locus. Initially, the central spot (20 × 20) was covered by plants homozygous for the marker gene while all surrounding individuals were homozygous for a recessive allele at that locus. Additionally, a male-sterile plant was simulated every six nodes, resulting in a 29 × 29 grid. These male-sterile plants were also homozygous for a recessive allele at the marker locus (Fig. 1).

The density function of pollen dispersal (sensu Part 1985) was chosen to be a negative exponential, which is one of numerous functions found in the literature (Okubo and Levin 1989). The probability that a pollen grain emitted by a plant of coordinates (x, y) falls on a plant of coordinates (x', y') could therefore be expressed as $f(x, y, x', y') = (\lambda^2/2\Pi)\exp(-\lambda\sqrt{(x-x')^2-(y-y')^2})$. The proportion of pollen travelling long distances decreases when λ increases. λ was fixed to 0.1 in the example described hereafter. This function verifies $\int_0^\infty 2\Pi r f(r)\,dr = 1$ where r is the distance between the two plants (Peart 1985).

The pollen dispersal was simulated as an isotropic random process. All male-sterile plants were considered successively, and for each of them a pollen donor was chosen randomly on the grid among male-fertile plants with a probability decreasing with distance along $f(x, y, x', y')$. The fact that fathers outside the field were not considered is consistent with the fact that all pollen grains came from the field and, reciprocally, that all pollen grains fell inside the field.

For each repeat, we simulated the fertilisation of exactly 2000 seeds per male-sterile plant. One hundred repeats were performed. For each of them, the respective proportions of marked and unmarked pollen grains that arrived on every male-sterile of the field were recorded. Provided that all pollen grains have a similar success, which is a reasonable assumption, this proportion is the same as that of marked individuals in their progeny, and we could thus derive the global pollen distribution as recorded in pollen-dispersal experiments.

Fig. 1 180 × 180 grid used to perform the simulations. The plants emitting marked pollen grow in the 20 × 20 central shaded square. They are surrounded by non-marked plants. The whole grid is covered by a 29 × 29 matrix of male-sterile receptor individuals



Edge effects

The finite size of the simulated field could have consequences at two levels. (1) Because no dispersal can occur at very long distances, we can expect each of the central male-sterile plants to be fertilised by pollen travelling shorter distances compared to what would be expected in an infinite field. (2) Because central plants have a higher number of neighbours than plants situated at the edge of the field, we can expect their contribution to the total pollen cloud to be larger than that of plants at field margins. This would also be true in a field experiment where part of the pollen from margin plants is lost outside the field.

Test of the method

Contrary to experimental data in which only the dispersal from the whole source plot ($g*f$) and the proportion of marked pollen produced (g) would be known, we here also know the f function. In order to check if the deconvolution of $g*f$ sucessfully provides this f function, we therefore compared two individual dispersal curves for each of the 100 repeats. The first (subsequently, the observed individual dispersal) is that actually achieved in a simulation. It is the curve which could not be recorded experimentally and which we would aim at determining. The second (the calculated individual dispersal) is that obtained by the deconvolution of the global dispersal function. It could be calculated from experimental data and should be a good approximation of the first. The similarity of these two curves would validate the use of the method.

The observed individual dispersal for each repeat was obtained by considering the origin of each of the 2000 pollen grains falling on each of the nine male-sterile individuals situated in the middle of the grid and thereafter averaging the nine curves thus obtained. This provided the proportion of pollen travelling each distance in every direction on a total of 18 000 pollen grains per repetition (the f function). The choice of the nine central male-sertile individuals was justified by the need to represent equally every direction without excessively increasing computing time. It maximised the probability of observing an excess of short distances in the distribution of distances travelled by pollen since these are the plants for which the maximum possible distance travelled is the smallest.

The proportion of pollen falling at every distance from the source was calculated by pooling results over distance for the observed and the calculated individual dispersals in each of the 100 simulations and their similarity tested by a t-test after arcsine transformation of the sq. rooted data.

Results

Observed individual pollen dispersal (f)

An example of the distribution of the origins of pollen grains falling on central plants from 1 of the 1000 repeats is presented in Fig. 2. When pooled over directions, the shape of the individual dispersal curve was very consistent among repeats (see standard deviations in Fig. 3).

Because the distance travelled by most pollen grains was short with respect to the size of the grid, we did not observe an excess of short-distance dispersal compared to that expected from the chosen negative exponential density function. This can be checked in Fig. 3 by comparing the distribution of pollen over distance in the 100 simulations performed on the 180 × 180 grid with that of a set of 10 simulations performed on a 360 × 360 grid.

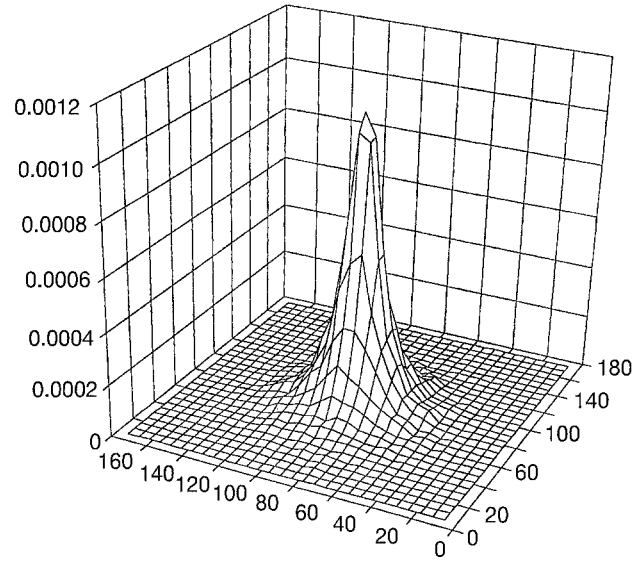


Fig. 2 Distribution of the origins of the pollen grains after a correction to centre it on the middle of the grid for 1 of the 100 repeats. This also represents the distribution of distances traveled by pollen grains in every direction (function f), i.e. the observed individual dispersal. (Obtained from the average of the nine male-sterile central plants, see text for details)

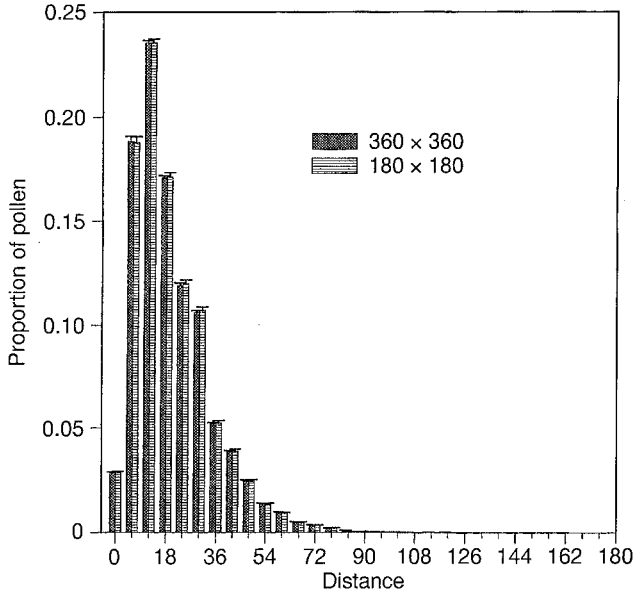


Fig. 3 Distribution of distances traveled by pollen grains falling on male-sterile plants pooled over directions in the 100 repeats on the 180 × 180 grid and in 10 repeats of the 360 × 360 control grid. *Bars* represent intervals of width 6. *Error bars* represent standard errors

Relative contribution of central plants to the pollen cloud

Figure 4 presents the average number of pollen grains emitted at each distance from the centre of the grid averaged over 100 simulations. The mean contribution to the total pollen cloud of a plant situated in the middle of the grid was larger than that of a plant situated near

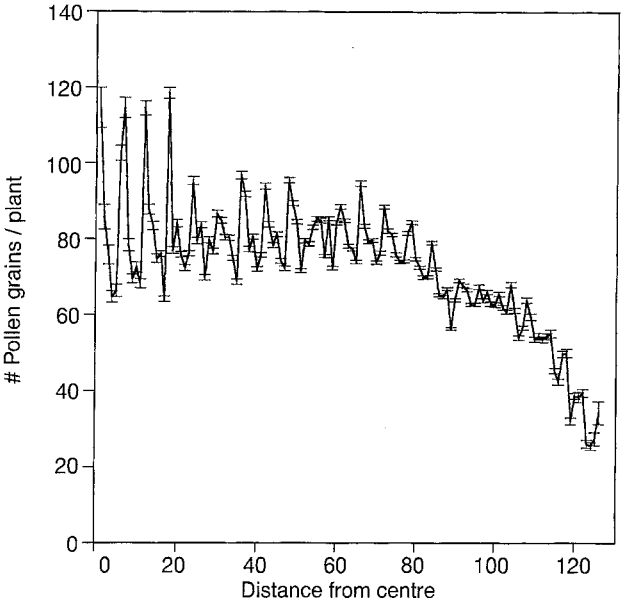


Fig. 4 Mean and standard error of the number of pollen grains per plant as a function of distance from the centre of the 180 × 180 grid in one simulation

an edge. This edge effect was most sensitive in the 20 most marginal rows of the grid. Because plants growing near male-sterile individuals contributed more to the pollen cloud that these individuals received, the curve also exhibits an oscillation of a shorter period. This oscillation follows the proportion of male-sterile individuals found at every distance from the source.

Global pollen dispersal ($g*f$)

For each repeat, global pollen dispersal was calculated as the proportion of marked pollen on each node of the grid after simulating the dispersal of 2000 pollen grains on each of the male-sterile receptor plants. The result of 1 of the 100 simulations is represented in Fig. 5. The curves were generally smooth, displaying little noise due to random events. When pooled over all directions, the proportion of marked pollen found on male-sterile plants decreased sharply with distance from the source, and almost no pollen from the source reached the edges of the grid as would be expected from the individual pollen dispersal (Fig. 6).

When the frequency distribution of the pollen emitted by the whole source (global) was pooled over all directions, it was very consistent among simulations (Fig. 7) and differed significantly from the frequency distribution of distances travelled by pollen grains (individual).

Calculated individual pollen dispersal

The deconvolution procedure was applied to each of the 100 global pollen-dispersal curves ($g*f$). The 100 calculated individual dispersal curves (f) thus obtained

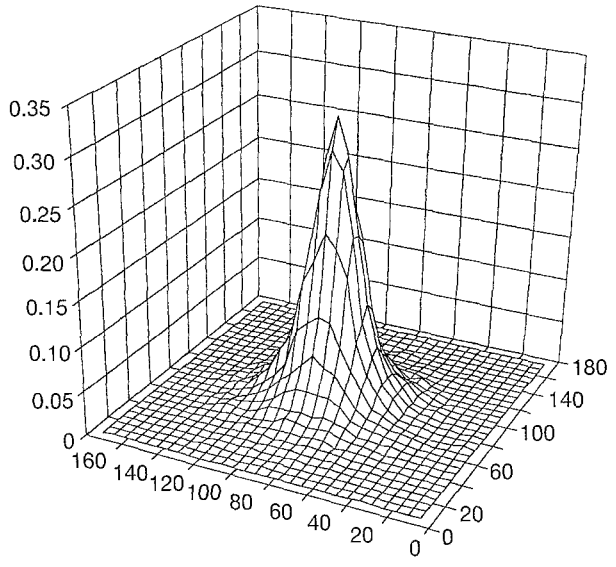


Fig. 5 Proportion of marked pollen among the total pollen received by each male-sterile plant on the grid for one simulation (function $g*f$)

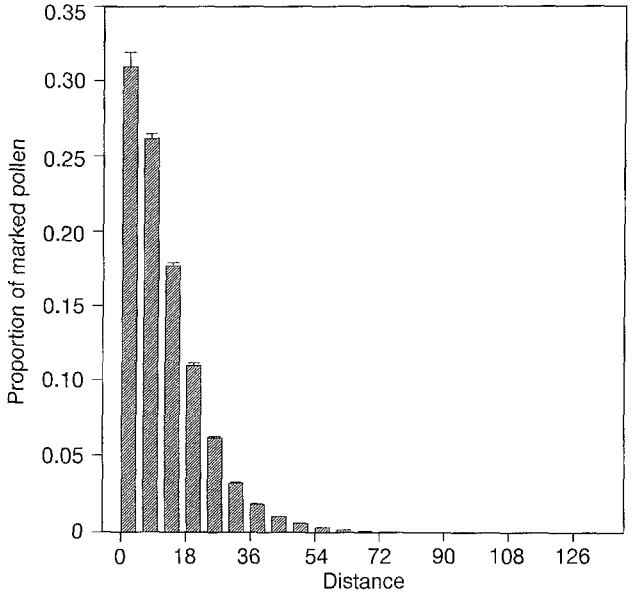


Fig. 6 Mean and standard error of the proportion of marked pollen among the total pollen received by male-sterile plants as a function of distance from the centre of the grid (100 repeats). Each *bar* represents the average for an interval of width 6

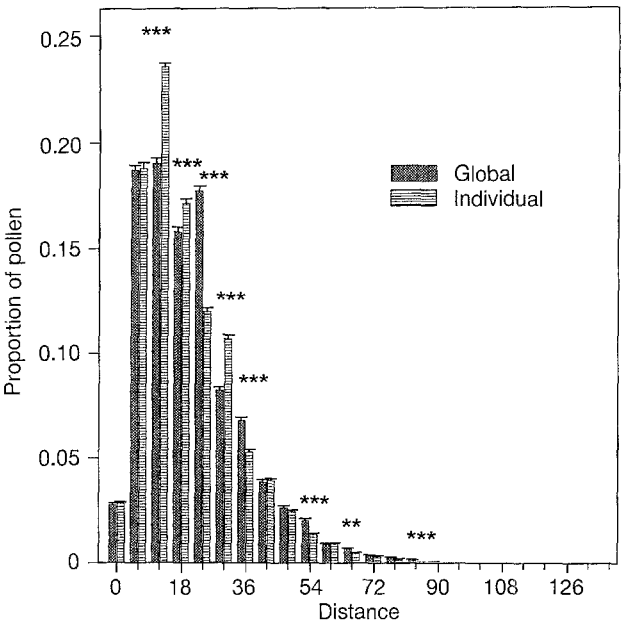


Fig. 7 Distribution of distances traveled by pollen grains, i.e. the proportion of the pollen emitted by an average plant that travels at each distance from this plant (individual) and proportion of the total marked pollen found at every distance from the centre of the grid (global). (Mean and standard errors from 100 repeats). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.0001$

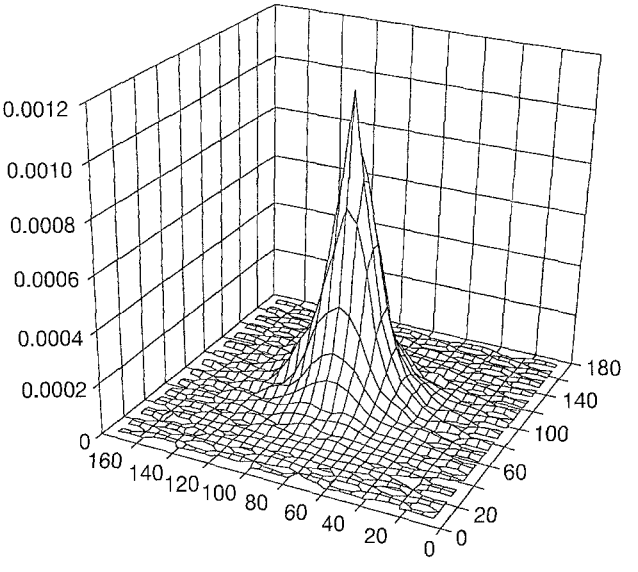


Fig. 8 Calculated individual dispersal corresponding to the observed individual dispersal presented in Fig. 2

presented oscillations due to the regular setting of the male-sterile receptor plants. These oscillations were flattened by taking, on each node, the average value of the node and its eight closest neighbours. As an example, Fig. 8 presents the calculated individual dispersal curve corresponding to the observed individual dispersal curve presented in Fig. 2.

When data from the calculated individual curves were pooled over directions, the resulting curve was very consistent among the simulations (see standard errors for the calculated dispersals in Fig. 9).

Test of the method

After values were pooled over all directions, the average calculated and observed individual dispersal curves were extremely similar. They both had values around

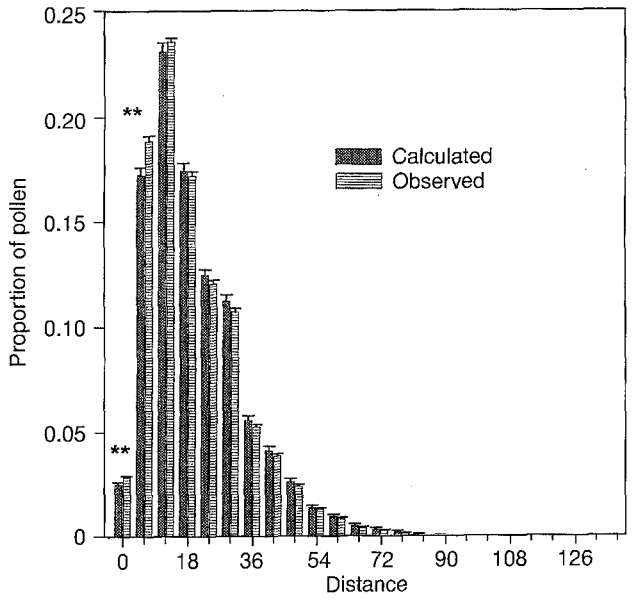


Fig. 9 Comparison of the observed and calculated individual dispersals averaged over 100 repeats on the 180 × 180 grid. (Mean and standard errors are given for intervals of width 6 around the source plant). ** $P < 0.01$

0.02 at very short distances, reached a peak around 0.23 for the distance interval (12, 18) and steadily decreased until a distance of 90 (Fig. 9). Although occasionally significant, the absolute value of the differences reached a maximum of 0.016 (interval (6, 12), a value well below the detection threshold in most pollen-dispersal experiments.

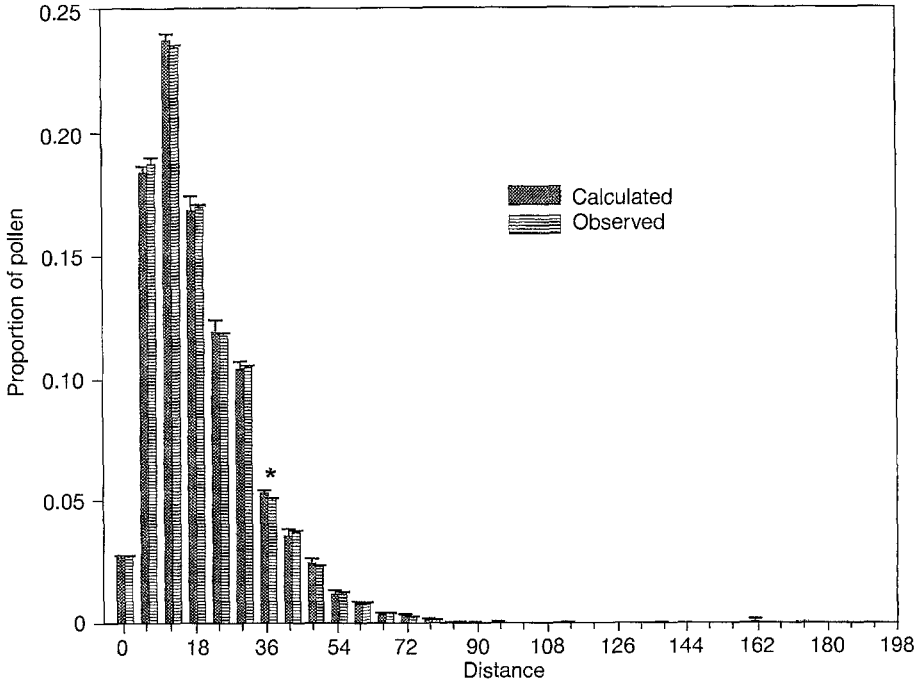
There was, however, a systematic bias in the sign of the difference. The proportion of pollen calculated to be falling at short distances was significantly smaller than that observed, and it was constantly (although not significantly) larger at medium to long distances (Fig. 9). This bias could be explained by the over-representation of plants carrying the marker gene in the pollen that travelled a medium distance due to edge effects; it disappeared when the edge effects were reduced by increasing the grid size to 360 × 360 cells (shown for ten repeats in Fig. 10).

Discussion

Pollen-dispersal experiments usually face the problem that a large source of pollen is needed to track rare long-distance dispersal events (e.g. Griffiths 1950; Levin and Kerster 1974; Scheffler et al. 1993; Skogsmyr 1994). Consequently, the observed dispersal is not that of one plant but the sum of those from all the source plants. It therefore depends on the shape of the source plot and can significantly differ from the dispersal of individual plants as demonstrated here (see Fig. 7).

The method we present is a possible way to determine the average pollen dispersal of a single individual from dispersal data using a large number of source plants. It has two main advantages: (1) it allows a quantification of long-distance pollen dispersal since the distribution obtained can be inferred from dispersal experiments that comprise a large source of pollen, and (2) it gives a pollen distribution that is independent of the shape of the pollen source. It can be applied to any pollen-dispersal experiment for which data are available concerning (1) spatial distribution of the plants emitting the marked

Fig. 10 Comparison of the observed and calculated individual dispersals averaged over ten repeats on the 360 × 360 grid. (Mean and standard errors are given for intervals of width 6 around the source plant). * $P < 0.05$



pollen grains; (2) the proportion of marked pollen grains produced by each plant (which is simply 0 or 1 when the plants are homozygotes); (3) the proportion of marked pollen falling on each recipient plant (which can be assessed by screening the progeny of the recipient plants if the marker is dominant). The distribution of recipient plants should be homogeneous but, contrary to what was done in the simulations described here, any periodicity should be avoided to prevent background oscillations.

The most critical assumption in the method presented here is that all plants disperse their pollen similarly and, in particular, that plants producing marked or unmarked pollen contribute equally to the pollen cloud; whereas random differences in dispersal among plants do not effect the outcome of the deconvolution (as shown by the result from the simulations on a 360×360 grid), a systematic bias in the relative contribution of the marked plants to the pollen cloud will lead to a bias in the results. This is because the proportion of marked individuals in the progeny of the recipient plants does not depend only on the distance to the source emitting the pollen carrying the marker gene but also on the contribution of the marked plants versus the unmarked ones at any given distance. This contribution depends both on the pollen production of the marked plants and on the distance of the receptor plant from the edge of the field.

This bias is, however, expected to be found in any experiment in which single plants carrying a marker gene are grown in stands of recipient plants and the pollen dispersal is inferred by screening the progeny. It can be avoided in field experiments by checking that source plants do not produce a significantly different amount of pollen or pollen that is more viable, and that the distance between the edge of the source patch and the edge of the field is at least twice as large as the distance within which a single plant is likely to disperse most of its pollen. This should not represent a strong constraint for most species.

Provided that some precautions are taken, the method described here could potentially be used in two kinds of ecological studies.

First, comparisons of the mean individual pollen distribution of a plant species (or of a crop variety) in different environments and/or different experimental designs would demonstrate the impact of environmental conditions on gene dispersal by pollen. Factors of interest which could be manipulated are, for example, patch size, plant density or aggregation, pollinator availability... Conversely, the comparison of different species (or varieties) in similar environments would give an estimate of the variability that exists on the shape of the pollen dispersal. Most field studies investigating the effect of environment on the pollen-mediated gene dispersal of individual plants have, until now, inferred gene flow from the movement of the pollen itself using microtags (Nilsson et al. 1992), radioactive tracers (Schlising and Turpin 1971; Massaux et al. 1976) or colour polymorphism (Thomson and Thomson 1989), from the movement of fluorescent dyes (Linhart et al. 1987; Campbell and Waser 1989) or from pollinator flight distances (Levin and Krester 1969). Using genetic markers, Campbell (1991) showed, however, that estimates of pollen movement tend to underestimate the actual movement of genes. This was confirmed by a description of gene movements within populations using paternity analysis studies (Godt and Hamrick 1993). This more recent method also provides a good estimate of the effective pollen dispersal of individual plants. However, in order to unambiguously determine the paternity of the seeds, this type of approach relies on the use of a large number of polymorphic genetic markers. This could create problems for the determination of the pollen distributions from a large number of plants, which would be necessary to quantify long-distance dispersal. It is also inappropriate for the many crop varieties that are characterised by low levels of polymorphism. As with paternity analysis, our method would necessitate the screening of a large number of progenies but only for a few marker genes as long as the distribution of the parental plants carrying these genes is known in the population. Each marker gene would provide an estimate of the frequency distribution of distances travelled by pollen grains. The advantage over paternity analyses is that the existence of plants dispersing similar pollen increases the precision of the estimates. Its drawback is that individual variation is not so precisely estimated. Moreover, the precision of the method when source plants are not grouped might need to be tested.

Secondly, and coming back to the risk assessment of the deliberate release of transgenic crops for which this method was originally designed, the individual pollen dispersal curve can be used as a basis to model dispersal from different shapes of transgenic fields or, for a given shape, to determine the contribution of edge plants to the pollen escaping outside the field, thus, for example, providing information on the likely efficiency of growing different widths of non-transgenic crops catchers around transgenic fields. This will help reduce the probability of hybridisation between transgenic crops and wild related species growing in the vicinity of the field.

Gene flow is one of the major processes determining the genetic structure of plant populations. Many more empirical studies will be needed before we understand the ways in which seed and pollen flow shape the distribution of alleles in natural and artificial populations. This method, together with other complementary approaches, such as paternity analysis, allows us to retrieve more information from the patterns of allele frequency observed in the field and therefore also improve the accuracy of existing models describing these systems.

Acknowledgments The authors wish to thank A. Genestier for all his ideas, M.L. Gauvin, C. Jost, D. Taneyhill and B. Schmid for discussions and A. McLellan and J. Shykoff for helpful comments on an earlier version of the manuscript. This study was supported in part by the EU BRIDGE programme by grant BBW 93.0271 and by ENSH for B. Godelle.

References

Bateman AJ (1947) Contamination in seed crops. II Wind pollination. Heredity 1:235–246

Bos M, Harmens H, Vrieling K (1986) Gene flow in *Plantago*. I Gene flow and neighbourhood size in *P. lanceolata*. Heredity 56:43–54

BRIDGE (1994) Safety assessment of the deliberate release of two model transgenic crop plants, oilseed rape and sugar beet. Final report

Calcote R (1995) Pollen source area and pollen productivity: evidence from forest hollows. J Ecol 83:591–602

Campbell DR (1991) Comparing pollen dispersal and gene flow in a natural population. Evolution 45:1965–1968

Campbell DR, Waser NM (1989) Variation in pollen flow within and among populations of *Ipomopsis aggregata*. Evolution 43: 1444–1445

Crawley MJ, Hails RS, Rees M, Kohn D, Buxton J (1993) Ecology of transgenic oilseed rape in natural habitats. Nature 363:620–623

Ellstrand NC, Devlin B, Marshall DL (1989) Gene flow by pollen into small populations: data from experimental and natural stands of wild radish. Proc Natl Acad Sci USA 86:9044–9047

Faegri K, Iversen J (1975) Textbook of pollen analysis 3rd edn., Blackwell Scientific Publ, Oxford London Edinburgh Melbourne

Galen C (1992) Pollen dispersal dynamics in an alpine wildflower, *Polemonium viscousm*. Evolution 46:1043–1051

Godt MJW, Harmick JL (1993) Patterns and levels of pollen-mediated gene flow in *Lathyrus Latifolius*. Evolution 7:98–110

Gregorius H-R, Steiner W (1993) Gene transfer in plants as a potential agent of introgression. In: Wöhrmann K, Tomiuk J (eds) Transgenic organisms. Birkaüser Verlag, Basel Switzerland, pp 83–107

Griffiths DJ (1950) The liability of seed crops of perennial ryegrass (*Lolium perenne*) to contamination of wind-brone pollen. J Agric Sci 40:19–38

Honig MA, Linder HP, Bond WJ (1992) Efficacy of wind pollination: pollen load size and natural macrogametophyte populations in wind-pollinated *Staberoha banksii* (Restionaceae). Am J Bot 79:443–448

Levin DA, Kerster HW (1969) The dependence of bee-mediated pollen and gene dispersal upon plant density. Evolution 23:560–571

Levin DA, Kerster HW (1974) Gene flow in seed plants. Evol Biol 7:139–220

Linhart YB, Busby WH, Beach JH, Feinsinger P (1987) Forager behavior, pollen dispersal, and inbreeding in two species of hummingbird-pollinated plants. Evolution 41:679–682

Luby JJ, McNicol RJ (1995) Gene flow from cultivated to wild raspberries in Scotland: developing a basis for risk assessment for testing and development of transgenic cultivars. Theor Appl Genet 90:1133–1137

Manasse RS (1992) Ecological risks of transgenic plants: effects of spatial dispersion on gene flow. Ecol Applic 2:431–438

Massaux F, Tchiendji C, Misse C, Decazy B (1976) Etude du transport du pollen de cacaoyer par marquage au 32P. Café Cacao Thé 20:163–171

McPartlan HC, Dale PJ (1994) An assessment of gene transfer by pollen from field-grown transgenic potatoes to non-transgenic potatoes and related species. Transgenic Res 3:216–225

Morris WF, Kareiva PM, Raymer PL (1994) Do barren zones and pollen traps reduce gene escape from transgenic crops? Ecol Applic 4:157–165

Nilsson LA, Rabakonandrianina E, Pettersson B (1992) Exact tracking of pollen transfer and mating in plants. Nature 360:666–668

Okubo A, Levin SA (1989) A theoretical framework for data analysis of wind dispersal of seeds and pollen. Ecology 70:329–338

Peart DR (1985) The quantitative representation of seed and pollen dispersal. Ecology 66:1081–1083

Press WH, Teukolsky SA, Vetterling WT, Flannery BP (1992) Numerical recipes in C: the art of scientific computing, 2nd edn: Cambridge University Press, Cambridge, pp. 496–545

Schaal BA (1980) Measurements of gene flow in *Lupinus texensis*. Nature 284:450–451

Scheffler JA, Parkinson R, Dale PJ (1993) Frequency and distance of pollen dispersal from transgenic oilseed rape (*Brassica napus*). Transgenic Res 2:356–364

Schlising RA, Turpin RA (1971)) Hummingbird dispersal of *Delphinium cardinale* pollen treated with radioactive iodine. Am J Bot 58:401–406

Skogsmyr I (1994) Gene dispersal from transgenic potatoes to conspecifics: a field trial. Theor Appl Genet 88:770–774

Stewart SC (1994) Simultaneous estimation of pollen contamination and pollen fertilities of individual trees in conifer seed orchards using multilocus genetic data. Theor Appl Genet 88:593–596

Stone R (1994) Large plots are next test for transgenic crop safety. Science 266:1472–1473

Tauber H (1965) Differential pollen dispersion and the interpretation of pollen diagrams. Geol Surv Denmark II Ser No 89

Tiedje JM, Clowell RK, Grossman YL, Hodson RE, Mack RN, Regal PJ (1989) The planned introduction of genetically engineered organisms: ecological considerations and recommendations. Ecology 70:298–315

Thomson JD, Thomson BA (1989) Dispersal of *Erythronium grandiflorum* pollen by bumblebees: implications for gene flow and reproductive success. Evolution 43:657–661

Tonsor ST (1985) Leptokurtic pollen-flow, non leptokurtic gene-flow in a wind-pollinated herb, *Plantago loceolata* L. Oecologia 67:442–446

Tynan JL, Williams MK, Conner AJ (1990) Low frequency of pollen dispersal from a field trial of transgenic potatoes. J Gen Breed 44:303–306

Umbeck PF, Barton KA, Nordheim EV, McCarty JC, Parrot WL, Jenkins JN (1991) Degree of pollen dispersal from a field test of genetically engineered cotton. J Econ Entomol 84:1943–1950

Wanger DB, Allard RW (1991) Pollen migration in a predominantly self-fertilizing plants: barley. J. Hered 82:302–304

Waser NM, Price MV (1982) A comparison of pollen and fluorescent dye carry-over by natural pollinators of *Ipomopsis aggregata* (Polemoniaceae). Ecology 63:1168–1172

Wright S (1931) Evolution in Mendelian populations. Genetics 16:97–159