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Using seed purity data to estimate an average pollen mediated gene flow from crops to wild relatives

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Abstract Gene flow from crops to wild related species has been recently under focus in risk-assessment studies of the ecological consequences of growing transgenic crops. However, experimental studies addressing this question are usually temporally or spatially limited. Indirect population-structure approaches can provide more global estimates of gene flow, but their assumptions appear inappropriate in an agricultural context. In an attempt to help the committees providing advice on the release of transgenic crops, we present a new method to estimate the quantity of genes migrating from crops to populations of related wild plants by way of pollen dispersal. This method provides an average estimate at a landscape level. Its originality is based on the measure of the inverse gene flow, i.e. gene flow from the wild plants to the crop. Such gene flow results in an observed level of impurities from wild plants in crop seeds. This level of impurity is usually known by the seed producers and, in any case, its measure is easier than a direct screen of wild populations because crop seeds are abundant and their genetic profile is known. By assuming that wild and cultivated plants have a similar individual pollen dispersal function, we infer the level of pollen-mediated gene flow from a crop to the surrounding wild populations from this observed level of impurity. We present an example for sugar beet data. Results suggest that under conditions of seed production in France (isolation distance of 1,000 m) wild beets produce high numbers of seeds fathered by cultivated plants.

Keywords *Beta vulgaris* · Transgenic · Pollen dispersal · Sugar beet · Gene flow · Crops · Risk assessment

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Introduction

Two main agronomic and ecological issues on the large-scale cultivation of transgenic crops are the likelihood and consequences of the transfer of transgenes through pollination to other fields, in which they would be undesirable, or to wild relatives of the crops (Raybould and Gray 1993; Dale 1994; Darmency 1994). Most crops have the potential to hybridise with wild related species. Hybrids have been successfully obtained in diverse genera such as *Brassica* (Kerlan et al. 1992; Darmency 1994; Baranger et al. 1995; Lefol et al. 1996), *Raphanus* (Klinger et al. 1991) or *Beta* (Santoni and Berville 1992; Boudry et al. 1993), *Helianthus* (Linder et al. 1998) and in many cereals (Doebley 1990; Till-Bottraud et al. 1992; Arriola and Ellstrand 1996). If a crop and a wild relative coexist, the frequency of hybridisation under natural conditions, however, will strongly depend both on their relatedness and on the pattern of pollen dispersal. A quantitative estimate of pollen dispersal will also prove necessary to quantify the quantity of genes that migrate between fields. For this reason, there has recently been a renewed research interest for the study of pollen-mediated gene flow.

A first approach is the direct modelling of pollen dispersal based on experimental data. One problem with pollen-dispersal experiments addressing this issue is that they are spatially and temporarily limited. Experiments with major crops such as oilseed rape (Scheffler et al. 1993), potato (McPartlan and Dale 1994) or cotton (Umbeck et al. 1991; Llewellyn and Fitt 1996) are typically conducted on fields of about one-hectare (1-ha) over one season. Methods are being published for the extrapolation of their results to larger distances. This

makes it possible to model situations where two adjacent fields are grown with two varieties of the same crop, or where wild plants grow within a field (Lavigne et al. 1996, 1998; Tufto et al. 1997; Gliddon 1999). This modelling is possible because the canopy is continuous and conditions of pollen dispersal can be supposed to be homogeneous. However, the modelling of pollen-mediated gene flow over a discontinuous landscape with roads, edges and fields grown with various crops is still a problem. Furthermore, a year to year variation in the intensity and patterns of pollen-mediated gene flow is likely to influence, for example, the fate of a transgene in wild populations (Morris et al. 1994; Stone 1994). Not enough experimental data are available to calibrate the models under different conditions, and this between year variation cannot yet be included in pollen-mediated gene-flow models. Physical models also exist that address the question of the transport of pollen by wind under different climatic conditions (McCartney 1990; Di-Giovanni and Kevan 1991). Their translation to effective gene flow, which depends on the biology of the crops, possible pollinators and the viability of the pollen, however, remains to be done. Modelling approaches that could predict the fate of a transgene over an agricultural landscape are therefore not yet available.

Other approaches to address the question of gene flow from crops to their wild relatives are based on the investigation of populations of the wild species. Such unvestigations cannot always distinguish between seed and pollen-mediated gene flow, although this is possible if cytoplasmic markers are used (e.g. on beet, Boudry et al. 1993). Gene flow from crops to wild relatives was directly demonstrated under natural conditions by observing morphological traits (Luby and McNicol 1995 on raspberry) or analysing neutral molecular or isozymic markers with alleles specific to the crop. Using the latter, gene flow was demonstrated for example on temperate crop species such as Alfalfa (Jenczewsky et al. 1999), Beets (Santoni and Berville 1992; Bartsch et al. 1999a, b), Foxtail millet (Till-Bottraud et al. 1992) and Sunflowers (Whitton et al. 1997; Linder et al. 1998). These approaches demonstrate the existence of a flow of cultivated genes into wild species but only quantify its cumulative effect over years. The survey of wild populations to observe rare cultivated markers furthermore requires a large number of samples and analyses. More indirect approaches based on population structure also exist for the estimation of yearly migration rates among populations. These approaches are based on the analyses of allelic frequencies for neutral markers. There are mainly two types of analyses based either on the genetic differentiation of populations as measured by Wright's Fst parameter or on the frequency of private alleles in populations (Slatkin 1985). However, the equilibrium assumptions of the models underlying such methods make them inappropriate for the quantitative estimate of the rate of gene flow from crops to their wild relatives. First, since crops are sown every year in different quantities and on different plots, migration rates are likely to vary largely between years. Moreover, the varieties grown can change from year to year, which makes the equilibrium assumptions unrealistic. Finally, populations of wild relatives of crops are represented by individuals distributed more or less continuously across space and are far from being discontinuous, more or less panmictic, units. The last point was addressed by Tufto et al. (1996, 1998) who designed a maximum-likelihood method to estimate migration matrices over a metapopulation. However, their method assumes that populations remain situated at the same location, an assumption unrealistic when crops are considered.

We present here a new method to estimate gene flow from crops to their wild relatives. Contrary to the approaches described above, this method makes no assumption about the spatial distribution of populations or equilibrium conditions. Its originality is that to estimate gene flow from crops to their wild relative it uses data on the inverse gene flow, i.e. from the wild flora into a crop species. These data are much easier to obtain because seeds of crop plants are numerous, easy to access and the parental genotypes are usually well known.

A method to estimate pollen-mediated gene flow from crops to surrounding wild relatives

We assume that wild and cultivated plants all have the same individual pollen dispersal function, γ, defined as the probability for a pollen grain produced by a plant situated at co-ordinates (0,0) to fall on a point of co-ordinates (x, y). γ is a two-dimensional density function such that $\int_{IR^2} \gamma(x,y)\,dxdy = 1$ where IR is the set of real numbers (Lavigne et al. 1996, 1998). This γ function is also referred to as the dispersal kernel in some ecological studies (Kot et al. 1996; Clark 1998). It does not depend on the number, density or location of donor and recipient plants, contrary to the function describing the level of cross-pollination for which it is often mistaken. The assumption that γ is the same for wild and cultivated plants is supported if both species have the same mode of pollination, approximately the same phenotype and grow in similar habitats. This would be more-easily realised with conspecifics or closely related species. In insect-pollinated species, plant density may indirectly influence the shape of the γ function by modifying the behaviour of pollinators. This assumption is therefore more likely to be true for wind-pollinated species.

We further suppose that dispersal is symmetric, i.e. the shape of the probability function is the same in opposite directions, with $\gamma(x,y) = \gamma(-x,-y)$. This would, in particular, be the case if dispersal were isotropic. For our purpose, it is similar to assuming that the distribution of wild and cultivated plants is not related (statistically) to the main wind direction (i.e. for example, all wild plants are not down wind to cultivated plants) or that one of the two species is not more attractive to pollinators.

The dispersal of pollen from all wild plants (from a set W of area $|W|$) on a point of co-ordinates (x,y) can be described by the probability density function:

$$f(x,y) = \frac{1}{|W|}\int_W \gamma(x-x', y-y')\,dx'dy'. \quad (1)$$

This function integrates to 1 over IR^2. Assuming that pollen production per unit area of wild plants is homogeneous, f is the convolution product of γ by the function describing the localisation of wild plants (Lavigne et al. 1996, 1998) normalised by the total area of wild plants. The probability for a pollen grain from a wild plant to fall on the set of cultivated plants (named C) can therefore be written as the sum of f over C, i.e.

$$m_{w\to c} = \int_C \frac{1}{|W|} \int_W \gamma(x-x', y-y')\, dx'dy'\, dxdy. \quad (2)$$

This is the emigration rate from W to C.
This is also:

$$m_{w\to c} = \frac{|C|}{|W|} \int_W \frac{1}{|C|} \int_C \gamma(x-x', y-y')\, dxdy\, dx'dy', \quad (3)$$

where $|C|$ is the area covered by cultivated plants.

Because wild and cultivated plants are assumed to have the same symmetric individual dispersal γ, Eq. 3 can be written as:

$$m_{w\to c} = \frac{|C|}{|W|} m_{c\to w}. \quad (4)$$

The emigration rate from the wild area to the cultivated area is therefore proportional to the emigration rate from the cultivated area to the wild area in such a way that:

$$m_{w\to c}|W| = m_{c\to w}|C|. \quad (4')$$

If, for example, the area covered with wild plants $|W|$ decreases, everything else being the same, the emigration rate from C to W, $m_{c\to w}$, will also decrease since less area of receptor plants will be available.

Observations: pollution of cultivated seeds with wild pollen

Data from pollen-dispersal experiments or from analyses of the purity of crop seeds classically report the observed proportion P_{obs} of seeds fertilised by wild pollen in the progeny of cultivated plants. This, in population genetics, would be the migration rate of wild pollen. Using the notations above, it can be written as:

$$P_{obs} = \frac{h_{wc} r|W| m_{w\to c}}{h_{wc} r|W| m_{w\to c} + |C| m_{c\to c}}, \quad (5)$$

where r is the production of pollen of a unit area of wild plants relative to that of a unit area of cultivated plants, h_{wc} is the success of a pollen grain of a wild plant compared to that of a cultivated plant on a 'cultivated' ovule, and $m_{c\to c}$ is the proportion of pollen produced by cultivated plants that falls on cultivated plants.

Prediction: pollution of wild plants with cultivated pollen

Our goal is to predict the inverse migration rate, i.e. the proportion of wild seeds fertilised by pollen from cultivated plants. Similarly to Eq. 5, it can be written as

$$P_{pred} = \frac{h_{cw}|C| m_{c\to w}}{h_{cw}|C| m_{c\to w} + r|W| m_{w\to w}}, \quad (6)$$

where h_{cw} is the success of a pollen grain of a cultivated plant compared to that of a wild plant on a 'wild' ovule, and $m_{w\to w}$ is the proportion of pollen produced by wild plants that falls on wild plants.

Deriving P_{pred} from P_{obs}

We shall assume for simplification that $m_{c\to c}$ and $m_{w\to w}$ are close to 1, i.e. most pollen produced by the fields (respectively the wild populations) remains in the fields (respectively in the wild populations). This assumption is supported by the usual shape of pollen dispersal curves, with most pollen falling within short distances of the source (Bateman 1947; Levin and Kerster 1974).

If the area covered with cultivated plants is much larger than the area covered with wild plants, which is usually the case when crop fields are large, then equations 4, 5 and 6 lead to the following approximation:

$$P_{pred} = \frac{h_{cw}|C| P_{obs}}{h_{cw}|C| P_{obs} + r^2 h_{wc}|W|}. \quad (7)$$

If the area covered with cultivated plants is smaller or in the same range as the area covered with wild plants, then equations 4, 5 and 6 lead to the following approximation:

$$P_{pred} = \frac{h_{cw}|C| P_{obs}}{r^2 h_{wc}|W|}. \quad (7')$$

The number of seeds fathered by cultivated crops predicted to be found in wild populations after a season of reproduction can moreover be written as

$$N_{pred} = s\, N_w\, P_{pred}, \quad (8)$$

where s is the number of seeds of a wild plant and N_w is the number of wild plants present around the fields (which are assumed to be responsible for P_{obs}).

Numerical applications to beet data

In the following we shall consider the theoretical case of seed production of a new variety of transgenic sugar beet, assuming current conditions for seed production. Our aim is to predict the number of transgenes that would migrate from an average 1-ha field of seed production to wild or feral beets using data on the proportion of cultivated seeds pollinated by wild beets.

Sugar beet (*Beta vulgaris* ssp. *vulgaris* L., Chenopodiaceae) is a biennial species grown for its root, from which sugar is extracted. Pollen is mainly dispersed by wind, but also by insects (Bateman 1947; Free and Williams 1975). In France, sugar beet has two main wild relatives with which it hybridises: *Beta vulgaris* ssp. *maritima* along the northern, western and mediterranean coasts, and a feral form of *Beta vulgaris*, in the South (Longden 1976; Boudry et al. 1993). In contrast to the cultivated sugar beet, the feral form is mostly annual. Its ability to flower without vernalisation is due to the major dominant bolting allele B (Boudry et al. 1994). Gene flow from cultivated to wild beets has been reported in France, as detailed below, but also in Italy (Bartsch and Schmidt 1997), in the United Kingdom (Raybould and Gray 1993) and in the United States (Bartsch et al. 1999a).

In 1998 in Southern France, seed production covered an area of roughly 3,200 ha and about 598 kg of seeds (approximately 70% of the European seed production) were harvested this same year (GNIS 1998). To ensure seed quality, conditions for seed production are that 10 years must separate two plantings of beet seeds on the same field, and all wild or feral beets within 1,000 m around of the fields must be destroyed. Nevertheless, feral beets, most of them annual, are sometimes observed around seed-production fields and a proportion of harvested seeds that results from accidental pollination by feral beets (Boudry et al. 1993; Raybould and Gray 1993). Hybrids can be easily recognised later since they flower in fields cultivated for roots. Estimates of pollution vary between 0 and 0.169% (mean over varieties of 0.04%) (ITB 1999). This estimate is corrected for the fact that late cold Springs can induce some flowering in the absence of the B gene.

To predict the number of transgenes that would migrate from a field of 1-ha of cultivated beets to wild beets in such a situation, we set the following conditions.

(1) Wild beets are homozygous for allele B. The justification for this assumption is that the proportion of annual plants in the feral populations is close to 90% (Desplanque et al. 1999a, b) and the B allele is indirectly selected by seed producers in feral plants because plants that do not flower the first year are more likely to be destroyed.
(2) Seed production design is classical with 70,000 plants/ha of which 20% are hermaphrodites, i.e. they produce pollen (N_c=14,000 plants per ha). The others are male-sterile.
(3) h_{cw}=h_{wc}=1, i.e. there are no hybridisation barriers between the wild and cultivated forms (Raybould and Gray 1993; Van Raamdonk and Schouten 1997).
(4) Wild plants produce 1,000 seeds per plant (s=1,000) (Desplanque et al. 1999b). This only modifies the predictions for the number of transgenic seeds found in wild beets but not for their proportions. Modifying s has a direct proportional effect on the predictions for transgenic seed numbers (Eq. 8).
(5) Each wild or cultivated plant covers the same area (i.e. they have approximately the same size).
(6) Since high pollen production is expected in hermaphrodites cultivated for seed production we considered two situations for pollen production: each wild plant produces as much pollen (r=1) as a cultivated plant or twice less pollen (r=0,5).

We simulated two situations, the first corresponding to the published level of cultivated seeds fathered by feral beets (i.e. P_{obs}=0.04%) (ITB 1999) and the second to a lower level (P_{obs}=0.01%) which would correspond to a higher purity of seeds, due for example to better weeding or larger isolation distances.

Given the observed levels of pollution in cultivated seeds, predictions can be made through Eq. 3 and 4 concerning the expected proportions and numbers of transgenes in the progeny of wild beets surrounding a cultivated field of 1-ha (Fig. 1). Since the number of wild plants responsible for the observed pollution is not known, these predictions are given for five hypothetical numbers of wild plants (from 10 to 1,000).

The pollution of cultivated seeds actually observed (P_{obs}=0.04%) can be explained by intense pollen-mediated gene flow from very few (N_w=10) wild plants or, at the opposite, by very low pollen exchange from numerous (N_w=1,000) wild plants. The predictions differ between these two situations. In the first situation the expected proportions of transgenic progeny for wild plants are large, but not for the numbers of transgenic seeds since the progeny are less numerous (P_{pred} about 0.35 and N_{pred} about 3,500 if both types of plants produce the same amount of pollen). In the second situation low proportions of transgenic progeny but high numbers of transgenic seeds are expected (P_{pred} about 5.10^{-3} and N_{pred} about 5,000 if both types of plants produce the same amount of pollen) (Fig. 1). If we assume that wild plants produce twice less-pollen (r=0.5) the differences between the two extreme situations become much larger

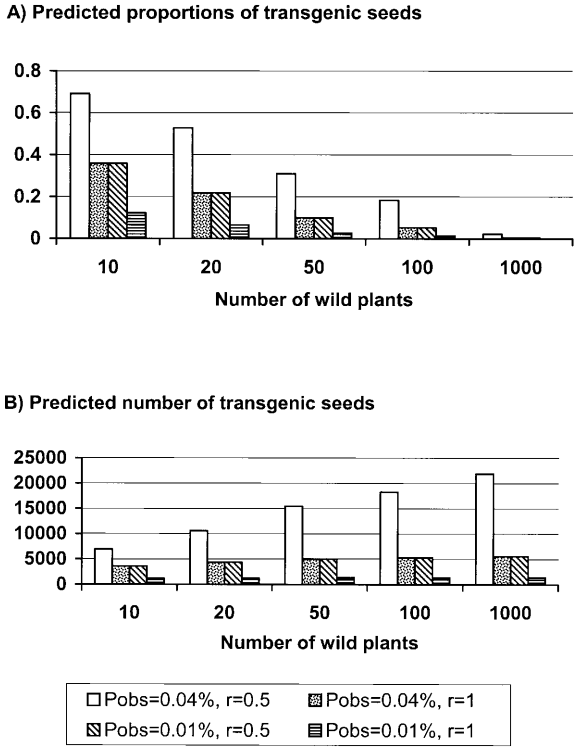

Fig. 1A, B Predictions of dispersal from the cultivated plants. Predicted proportion of transgenic seeds in the seeds of wild beets (**A**), and predicted number of transgenic seeds found in wild beet populations (**B**), for a field of seed production of 1-ha, two values of observed seed pollution (P_{obs} 0.01% and 0.04%), various numbers of wild plants responsible for this pollution (N_w from 10 to 1,000), and two values for the relative pollen production of wild plants (r 1 and 0.5). The results were obtained with equations 7 and 8

(P_{pred}=0.69 and N_{pred}=6,910 if N_w=10 and P_{pred}=0.022 and N_{pred}=21,900 if N_w=1,000).

The results are qualitatively similar if the observed proportion of cultivated seeds fathered by wild plants is lower (P_{obs}=0.01%) but all predicted values are reduced since gene exchanges are less intense. The decrease in P_{pred} is less than proportional to the decrease in P_{obs} (Fig. 1 and Eq. 6), meaning that reducing by a factor 4 the level of impurities in the seed crop would reduce less the proportion of transgenic seeds in the progeny of wild plants.

Discussion

We provide in this article a method to estimate the average quantity of seeds that would contain a transgene in a population of wild plants related to a transgenic crop after 1 year, using information about the pollution of fields by genes from wild plants. This information is easier to acquire than the direct measurement of pollution of wild plants because wild plants are often difficult to find, especially in areas of crop seed production where they are systematically destroyed when observed. Further-

more, because the genetic composition of most modern crop plants is usually known, it is possible to track events of pollution from other species. We chose sugar beet as an example for predictions because hybrids between cultivated and wild beets are easy to recognise due to their ability to flower without vernalisation (ITB 1999) and their proportions in commercialised seed lots are well established. The three main hypotheses of the method (similar wild and cultivated individuals distributed randomly with regard to main wind direction and most pollen falling within a short distance from the source) furthermore appeared reasonable; hybridisation between the wild and cultivated forms is easy and, despite the efforts of farmers, both forms coexist in the region of seed production (Boudry et al. 1993; Desplanque et al. 1999a). The predicted numbers of transgenes transferred from a 1-ha transgenic beet crop to seeds on wild relatives appear high for situations where many wild plants are responsible for the levels of hybrids actually observed among crop plants.

The figures provided by the method for the numbers of genes transferred are to be considered with care. First, the method only provides an average estimate for a 1-ha field and situations are likely to differ from field to field. The later fate of the seeds sired by cultivated plants will furthermore depend on their ability to reach maturity and to germinate successfully. A low survival to germination is expected, in particular when wild plants are destroyed by farmers, which will largely diminish the number of transgenic seeds in wild populations compared to our predictions. This low survival, however, should not modify our predictions concerning the proportion of transgenic seeds expected in wild populations, unless hybrids are little viable or the transgene has a negative impact on fitness. We therefore provide predictions for a potential, but not realised, migration rate. For a given immigration rate, the frequency of the transgene over years will furthermore depend on stochastic effects and on the relative fitness of transgenic individuals outside the field. This fitness will highly depend on the trait encoded by the transgene (Van Raamdonk and Schouten 1997; Hails 2000). The probability of fixation of a transgene that would be favoured outside the fields will furthermore depend on the number of wild plants, on their spatial distribution and on the probability of extinction of local demes (Barton and Whitlock 1997).

The predictions provided are valid for a given level of pollution P_{obs} in the crop. Obviously, if more-efficient weeding decreases the abundance of wild plants, this would also decrease the P_{obs} and therefore our estimates of gene flow.

Experimental studies assessing gene flow from oilseed rape crops in an agricultural landscape have demonstrated that various pollen sources (fields, but also feral populations) interact to determine the actual levels of gene flow, which are larger than what would have been predicted from single-field pollen-dispersal experiments (Squire et al. 1999; Thompson et al. 1999). With a very different approach, also situated in an agricultural landscape, we similarly provide results that predict the existence of a non-negligible pollen-mediated gene flow from cultivated to wild beets, although all wild beets are supposedly destroyed within 1,000 m from the fields. This conclusion appears inconsistent with results of pollen-dispersal experiments from single plots. Rüdelsheim et al. (1994) report dispersal mainly within 35 m but low frequencies at 100 m from the source. Bateman (1947) reports a faster decrease for red beet. These experimental results, however, cannot explain the observed level of pollution in the seeds of the crop. This is a typical example of the general discrepancy between the results on pollen movement provided by a ‘source’ (dispersal from a plot) and a ‘sink’ (search for non-self markers in isolated recipient plants) approach, where the ‘source’ approach tends to conclude that dispersal happens over shorter distances than the ‘sink’ approach (St Amand et al. 2000). Two non-exclusive particular mechanisms can explain this inconsistency: (1) wild beets may not be systematically destroyed by seed producers which would correspond to the situation where N_w=10, i.e. few wild beets growing close to the crop are responsible for the pollution, (2) the pollen-dispersal experiments set up so far may also largely underestimate the long-distance pollen dispersal in sugar beet and wild beets outside of the isolation area would be responsible for the pollution (this would correspond to N_w=1000). In any case, our results suggest that, as for oilseed rape, gene flow should be taken into consideration on the scale of the landscape.

The approach described in this article could also be adapted directly to cross-pollination between fields grown with different varieties (for specific production lanes) or to other biennial crop species with annual wild relatives such as carrots, chicory or lettuce, and more generally to crop species for which markers of gene flow from wild relatives are available.

In general its application would provide an average prediction regarding the potential dissemination of transgenes under realistic agricultural conditions. Obviously, this prediction is an average value and cannot be used to point out specifically which feral or wild population would be under more risk of receiving genes from crops. However, we believe that it is fruitful to compare and cross-validate different approaches to predict the level of gene flow from crops to wild related species, and we hope that this method will provide an additive tool for advisory committees assessing the ecological impact of transgenic crops.

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