Gene flow at the landscape level

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ABSTRACT

The distribution of fields and fragmentation of habitat in agricultural landscapes has a dominant effect on the rate of gene flow among crops and feral or wild relatives. An approach to quantifying the salient factors is illustrated with reference to experimental and theoretical studies of oilseed rape crops and feral brassicas. Existing models of long distance pollen movement underestimated gene flow when compared with measurements of pollen concentration and pollination in an agricultural context. The evidence indicates that large pollen sources, such as crop fields, interacted on a regional scale to increase gene flow. The value of modelling landscape-scale gene flow for synthesis and forming hypotheses is illustrated. Existing approaches, based on pollen-trap measurements from what were presumed to be single source fields need to be modified to account for multi-source interference.

INTRODUCTION

In the study of gene flow and its consequences for agriculture, the main feature of the landscape is the fragmented or distributed pattern of its crop fields and the associated populations of feral or wild relatives. This fragmentation is due in part to variation in the soil and topography, and in part to the variety of agricultural practice. Interactions both between and within fragments are important (Husband & Barrett, 1996). Movement of pollen and propagules determines the potential rate of spread of a gene, while local interactions for space or resources determine the rate at which individuals containing the gene increase in number or become extinct. While mechanisms of dispersal and pollination set the upper spatial limit to possible exchanges in any reproductive cycle, the local population dynamics determine the consequences of those exchanges.

However, the rate of gene transfer from one plant to another and the residence time of the gene in the population are unlikely to be independent factors. Suppose, for example, that a new genotype was grown in an agricultural region for a few life cycles and left a number of feral populations containing its genetic material. Any property or factor conferring stability on the individuals in a fragment would increase the residence time of the genetic material but tend to reduce its spread to new individuals within the fragment. Also, dynamics that maximise the rate at which a gene increases in frequency at the landscape scale are unlikely to coincide with *both* the maximum residence time in a fragment and the maximum rate of gene movement between fragments. Rather each would be in some optimum state. If gene flow is undesirable, knowledge of such optimum states should allow policy and management

to avoid them. The challenge is to define them from imperfect knowledge of processes operating over a wide range of spatial and temporal scales.

There is a need for both conceptual and mathematical frameworks to enable scaling between local and regional processes and to incorporate specific observational and experimental detail on factors such as the physiological properties of individuals, their competitive interactions, the mating system, competition between self and foreign pollen and the properties of the pollen vector. Research towards developing such a framework has been in progress for several years and is now summarised. Many of the issues considered are generic and will be widely applicable in open pollinated systems.

GENE FLOW IN FERAL BRASSICAS AS A MODEL SYSTEM

Crop fields of oilseed rape and feral descendants of these crops exist in close proximity throughout the arable areas of the UK. Here *feral* includes both volunteer weeds within fields and other populations on field margins, soil dumps and roadsides. The crops and ferals are mainly *Brassica napus*, accompanied in certain areas by crops and ferals of *Brassica rapa*, and by wild *Brassica rapa*. Ferals are a common and widespread weed in cereal rotations, anecdotal evidence in many areas indicating they reside for several years in the seedbank (Lutman, 1993). The commercial lifetime of most cultivars is only a few years, so many seedbanks are likely to contain the descendants of several cultivars. Within arable fields, the volunteers and crops mingle intimately, but the plant habit of the volunteers is still governed by the cycles of cultivation and spraying, so remains similar to what it was in the original crop. The feral populations outside fields experience a wide range of selection pressures, leading to diverse forms including individuals that flower when very small (<5 cm height) to those that flower in at least two successive years. Many of the out-of-field feral populations are not routinely controlled, while phenotypic plasticity allows others that are controlled to avoid or recover from cutting or herbicide treatment. Seed is spilled while in transport and moved around in farm machinery. Pollination can occur by both wind and insect but the extent of each is uncertain in the arable context. Potentially, therefore, the feral oilseed rape in a region can receive genetic material from crops, keep it for several generations, and pass it on to other crops and ferals even when the cultivar source is no longer extant.

Much of the evidence of population dynamics and gene flow in oilseed rape has been obtained in a context that could not directly inform regional scale studies of feral populations. So, for instance (Bilsborrow *et al.*, 1998; Scheffler *et al.*, 1993), estimates of rather low levels of gene flow from moderate-sized plots to a somewhat larger surrounding planted area were more representative of gene flow from a feral population to a nearby or surrounding crop than the reverse. On population establishment, the work of Crawley *et al.* (1993) on the dynamics of oilseed rape populations artificially established in a range of habitats was mostly relevant to the risk of oilseed rape invading semi-natural vegetation. The increasing evidence of the extensive persistence and long-distance gene flow among crops and feral populations has developed from three other lines of investigation, all carried out in an agricultural context. First, demographic surveys in areas of Tayside and north east Fife defined the density of fields and feral populations in the landscape (DETR, 1999). About 25% of the populations recurred at the same site over at least two years, while others disappeared for a year or more then reappeared. Since the recurring populations might have

been caused by new seed spilled each year, the second line of research developed DNA markers to distinguished previous and extant crop cultivars (Charters *et al.*, 1996). Use of these markers gave unequivocal evidence that a cultivar could persist for several years at a site, even after it had ceased to be grown in the area; that populations could consist of more than one cultivar; and that outcrossing occurred within feral populations and from crops to ferals, sometimes leading to a genetically heterogeneous feral population. In one population studied, the descendants of a crop cultivar have persisted for at least ten years. The third line of investigation on transects of pollen density and direct measurements of gene flow indicated that early work with small sources might have underestimated the distance of pollen movement (Timmons *et al.*, 1995). Although the evidence for actual hybridisation was still very sparse and uncertain at distances over 0.25 km, many feral populations in the region lived within the hybridisation range of at least one field.

The application of modelling

None of this work in isolation could inform the crucial question of what would happen if many transgenic fields were distributed in the landscape. Clearly, if pollen concentrations decay rapidly with distance, such that fields are (in effect) isolated from each other, then regional considerations are not important; but if the pollen fronts from fields coalesce, then landscape scale behaviour – which might be unpredictable from work with single, small sources - is possible. A preliminary landscape-scale model was therefore developed as a means of synthesis and hypothesis-forming (Crawford *et al.*, in press; Timmons *et al.*, 1996). Equations based on field data represented pollen movement by air and deposition with distance from a field to give a two-dimensional pollen density profile over a simulated landscape. Simple assumptions were that there was enough pollen to fertilise every flower, and that self and foreign pollen were equal in their efficacy to pollinate. The percentage hybridisation between, say, transgenics and ferals was simply in proportion to the relative concentrations of the two types of pollen. When transgenic and non-transgenic fields were distributed together with feral populations at realistic frequencies found in the demographic survey, landscape-scale behaviour was implicated, specifically in that the gene flow from transgenics and ferals was influenced by the regional aggregation pattern of transgenic and non-transgenic fields. Although the model predicted substantial hybridisation in some areas, there were still major uncertainties over the existence and effect of long distance airborne transport (>1 km), and the balance between wind and insects as pollen vectors.

The principal purpose of new theoretical and experimental work in 1998 was to test the hypothesis that the distribution of fields and ferals on a landscape scale plays a significant role in gene flow. An experiment was constructed in two parts, as described later in this volume (Thompson *et al.*, 1999). The first used male-sterile bait populations as biological pollen traps, glass slides covered in double-sided sticky tape to measure surface deposition of pollen and a series of pollen suction traps, measuring airborne concentrations, all distributed along a line from one relatively isolated field, starting in the field itself then at 5 m, 170 m and 400 m. The results from this transect would provide back-reference to previous pollen transects on which the model was based. In the second part, bait plants and sticky slides were distributed in the region during the flowering period of oilseed rape. The aim was to determine pollen transport parameters from the line transect, and use these to predict the distribution of aerial and surface deposited pollen. A comparison with the new regional-scale data would confirm or reject the hypothesis.
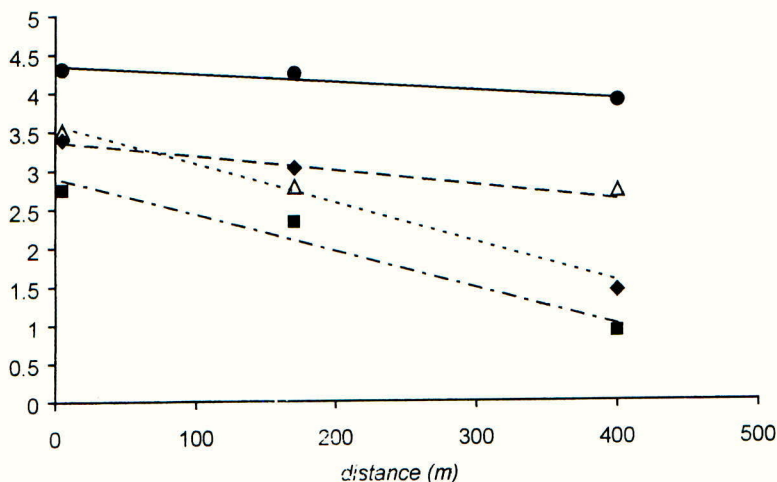
Figure 1. Plot of the logarithms of (filled squares) seed number produced per flower, (triangles) pollen concentration ($cm^{-2}d^{-1}$) on the glass slides, (diamonds) pollen concentration in arbitrary units recorded in suction traps, (circles) pods produced per flower i.e. % hybridisation, with distance from the field margin.

Clear trends were found in aerial and deposited pollen concentration, seeds/flower and % hybridisation with distance along the line transect (Fig. 1). A feature of the trends is the nature of the distance dependence of the airborne and deposited pollen concentrations. Both are consistent with an exponential decay with distance ($r^2=0.94$ and $r^2=0.72$ respectively). However the decay rate is different: the airborne pollen falls off more quickly with a characteristic dispersal distance, α_a, (equal to the distance at which pollen drops to $1/e=0.37$ of the value at the margin) of 200 m compared with the deposited pollen which has a characteristic dispersal distance, α_d, of approximately 500 m. The difference is most likely due to the fact that pollen is lost from the plume sampled by the traps both through deposition and upward convection. This confirms the need to understand the consequences of the sampling technique for the interpretation of measurements. The value of α_a is consistent with the values recorded in previous years in a different part of the region (Timmons *et al.*, 1996) but significantly larger than reported from plot experiments (e.g. McCartney & Lacey, 1991). Slide data collected from a similar transect adjacent to another field of oilseed rape in the current experiment showed the same spatial dependence and value for α_d. By contrast, slide data collected from a transect adjacent to a field of turnip rape (*B. rapa*) showed a lower value of α_d around 200m. Such large distances imply that many fields in the landscape will be in wind-borne pollen contact with feral populations.

As a first means of scaling between field and landscape, the existing model for airborne pollen transport (Timmons *et al.* 1996, Crawford, Squire & Burn, in press) was calibrated

from the transect containing the pollen traps. The model includes a term for deposition and therefore we could calculate both airborne and deposited pollen levels. However, the model was essentially two-dimensional and therefore did not include the effect of convected losses of pollen into the higher atmosphere. The level of sophistication required to deal with 3-dimensional transport is substantially greater and requires data collection of an intensity that is beyond the scope of regional-scale studies. By using our simpler model, we ignore upward convection and consequently $\alpha_a = \alpha_d$ and the value of α_d is underestimated. Predicted deposition over the landscape was therefore compared with actual measurement of pollen on the glass slides. Since it was not possible to independently determine a value for the pollen production rate from the fields, an arbitrary value was adopted. The resulting output from the model will properly predict the distance dependence in relative pollen levels, but not the absolute amounts.
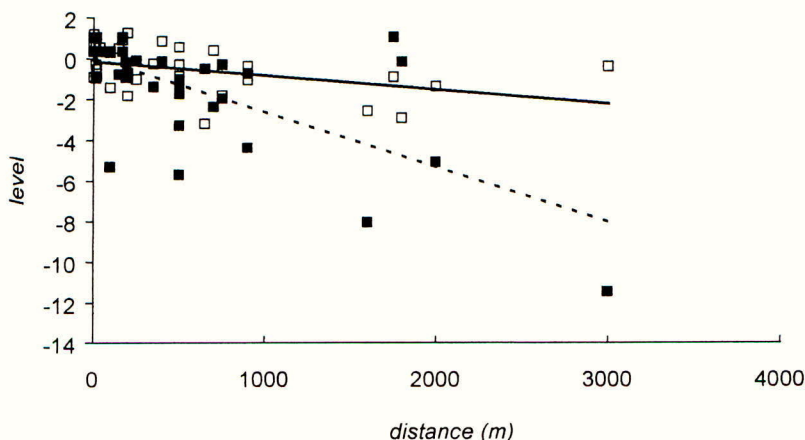

Figure 2. Plot of predicted (filled squares) and measured (open squares) levels (arbitrary units) of deposited pollen as a function of distance to nearest oilseed rape field (m). The absolute values have been renormalised for graphical clarity, a process that does not affect the slope of the distance relation.

Examples of the approach and indicative output (Fig. 2, Fig. 3) illustrate generic problems in scaling. Measured deposition was more variable at the landscape scale (Fig. 2). The value for α_d estimated from the slide data is 1500 m, three times larger than that estimated from comparable data on the transect. The measurements over a 400 m line extending from a single field were not therefore representative of pollen deposition over the landscape. The modelled values of deposition show even more variation between sites (Fig. 2); the value of α_d is 370 m, a similar order to that measured in the transects but about a quarter of that measured at the landscape scale. These results indicate the original hypothesis is correct: regional context has a significant role in determining the amount of pollen transferred by wind from fields to the environment. In calibrating our model, we chose a relatively isolated

field. However, until the results of the simulation and analysis were complete, we could only have provided a subjective and qualitative definition of 'isolated'. The transect probably suffered significant interference from surrounding fields (Fig. 3), even although these were the order of 1 km or more distant. Therefore our calibration was context dependent. The discrepancies between measured and modelled values appear to have a complex origin. As discussed above, the estimates at distance are very sensitive to the slope measured in the transect (Fig. 1), while the two-dimensional nature of our pollen transport model means that it will underestimate α_d. Furthermore, distance to the nearest field is not an appropriate variable at the landscape scale: only locations in the more isolated parts of the study area would have received wind-born pollen from mainly one source. The analysis shows the uncertainties that arise from scaling just one variable, airborne pollen. Scaling other salient factors, such as insect-borne pollen, would add further complexity.
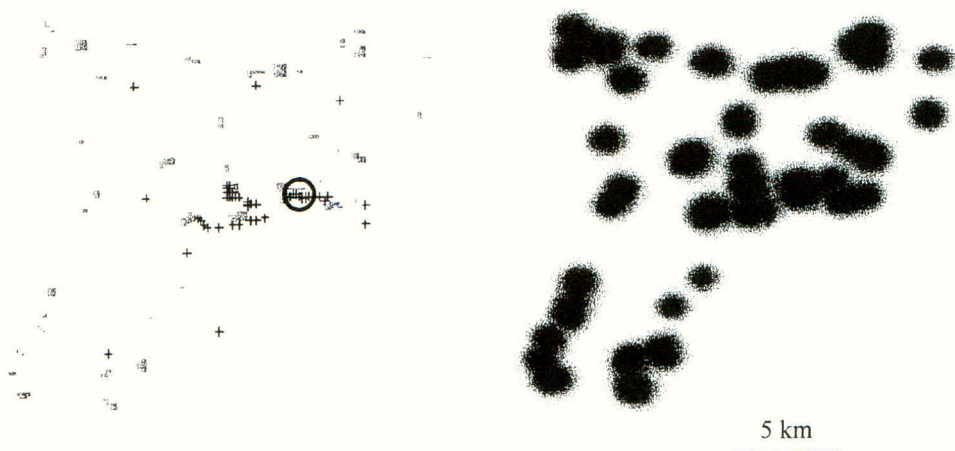

5 km

Figure 3. Left: map of fields (grey areas) in the 20 km x 20 km study region. Crosses indicate the locations of bait plants and glass slides for collecting deposited pollen. Circle identifies the main transect. Right: contour map of deposited pollen levels predicted by the model indicating the degree of mixing across the region after 2 simulated days of pollen release.

DISCUSSION

Crop and feral oilseed rape in a region such as Tayside should be considered as a metapopulation governed by a combination of local population dynamics at numerous sites, seed movement and gene exchange by pollen. Many other distributed, sexually compatible populations are likely to behave in a similar manner, differing only in degree from that of the feral brassicas. Therefore aspects of local stability and regional connectivity described here will be relevant to other systems. Further definition is required, both with respect to oilseed rape and generally, of both long-distance and local processes.

The effects of the spatial arrangement and size of fragments on pollen transfer are still uncertain and will be influenced by the type and features of the pollen vector as well as local landscape. For example, variation in pollination in the study with bait plants was traced, among other things, to local features such as woodlands and the occurrence of insect pollinators. Molecular techniques allow paternity to be identified in feral oilseed rape, but only where the pollen sources are sufficiently distinct. In some feral populations we studied, up to 50% of the progeny of some individuals contained genetic material that came from other plants as a result of outcrossing in that season. Given the landscape-scale gene movement from crops to ferals, however, several identical source cultivars will usually be within pollination distance of any feral population, making precise detection of the source impossible in many circumstances. Elsewhere, more definition has been obtained. In fragmented populations of the insect-pollinated tropical tree, *Swietenia humilis*, source-individuals were identified by paternally transmitted markers. Gene flow within moderate or large fragments was mainly between individuals in the fragment, while gene flow between fragments (up to 5 km) was greatest from small to large fragments (White & Powell, 1997). More generally, the relative extents and direction of between- and within-fragment gene flow might depend on the distance between fragments, the flight behaviour of the insect vector or aspects of the airborne pollen profile, as well as sexual compatibility. All are likely to be context-specific, but the possibility exists that a small degree of isolation within the metapopulation structure increases the likelihood of gene movement out of isolated populations.

The causes of establishment and spatial heterogeneity in the distribution of feral populations are uncertain. Areas of high occupancy by feral brassica populations are known to exist, partly because the crop is frequently grown there, but for other reasons that must favour the establishment of ferals. Such areas allow build up of populations from many different cropping and harvesting events, thereby allowing gene exchange over many years. Moreover, the stability over time of local populations has rarely been examined in detail, though great differences exist in their longevity. While the observational evidence for brassicas agrees with experiments that feral oilseeds are not becoming established in perennial scrub, grassland or woodland, evidence is accumulating that ferals can be stable and long-lived within and around the margins of agricultural land. Molecular techniques coupled with local knowledge have demonstrated persistence for over ten years in our study region. The contributions of source-cultivar physiology, and the effect of environment on local sorting and selection need to be much better defined.

The attempt to model regional gene flow has shown the need for a deeper understanding of the underlying principles, as opposed to a fragmentary and occasional look at topical issues. The purpose of modelling as used here is not so much prediction of absolute events but to produce testable hypothesis based on existing information, much of which is still at a small scale. Modelling and experimentation are therefore complementary and cyclical. Reaching an understanding of the broad picture will guard against unsound generalisations arising from specific experiments out of context.

ACKNOWLEDGEMENTS

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Genetic pollution: concepts, concerns and transgenic crops

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ABSTRACT

Genetic pollution implies the loss of identity of wild plant species as a result of transfer of genes from crop plants, with engineered fitness genes causing special concern. However, significant reproductive barriers exist between most wild species and crop plants. Gene flow is expected to have its greatest effect on weed species which are closely related to crop plants, taxonomically, ecologically and in their reproductive biology. Such plants may already have a long history of gene exchange with their crop relatives.

INTRODUCTION

Recently, conservation interest has become increasing focussed on the need to maintain biodiversity, as witnessed by a number of national and international political initiatives (the 1992 Rio Convention on Biological Diversity being the best known). This carries with it the implication that it is necessary to retain not only a range of habitats and species, but also the separate gene pools giving rise to the singularity of species and distinctive variants at the infra-specific level. Under this philosophy, individual, isolated, populations become worthy of conservation because their genetic structure endows them with a suite of characteristics which may be unique. It may also provide both short term fitness and long term evolutionary potential which, together, act as a buffer against extinction. The desire to maintain the maximum possible amount of genetic diversity has, in turn, given rise to fears that the genetic individuality of local populations or rare species with small populations could be compromised following gene flow from external sources. Consequently, the management of risk to genetic identity has become a component in the design of conservation strategies for rare species.

Genetic drift and, to a much lesser extent, mutation will tend to produce variation between populations which are small and isolated reproductively, so that those populations come to differ in morphology, physiology, biochemistry or in life history and breeding system characteristics. Gene flow, even at very low rates, will counter such differentiation. The greater the amount of gene flow between populations, the less the opportunity for the stochastic development of genetically distinct populations by drift. Where selection strongly outweighs the counterbalancing effects of gene flow, and dominates any tendency for genetic drift, it will dictate the evolution and genetic architecture of plant populations. It is the balance between these three processes which determines the genetic character of populations and any changes that may take place in them. It is against this background that genetic pollution risks must be assessed

WHAT IS GENETIC POLLUTION?

The term "genetic pollution" (Butler, 1994) is one of a number of disparaging terms which have been used to define the mixing of gene pools by hybridisation and introgression, especially where a common, non-local species or variety has been brought into contact with a small local population or a rare species. Less emotive are expressions such as, "genetic assimilation" (Ellstrand, 1992) and "mixing" (Rhymer & Simberloff, 1996). Whatever the terminology, the reference is to a single process which raises three fundamental problems having philosophical, ecological and practical implications:

- that locally-adapted populations, or whole species will become extinct
- that natural patterns of diversity will become obscured within a gene pool common to all populations of that species, and that this will ultimately compromise the capacity to adapt to environmental change
- that the introduction of new fitness traits will have long-term implications for population genetics and community dynamics.

The consequences of gene flow producing such effects has been voiced, particularly in relation to the use of non-local material in wildflower seed mixes (Akeroyd, 1994; Handel, *et al.*, 1994) but may also be invoked in considering the effects of forage or arable crop species on close relatives in natural grasslands.

Many of the terms used imply that there is an inherent value in what are regarded as "pure" gene pools and that this value is lost following introgression of genes from outside this pool. This view is incorporated in various national and international conventions whose underlying ethos is to protect variety, including genetic variation between and within species. The need for such protection of variation is rooted in a belief that it forms part of a national or international heritage and, as such needs to be regarded in much the same way as York Minster or the pyramids of Egypt. These buildings contain within their structures evidence of their history, their purpose and the culture of the societies which constructed them or modified the original design. In the same way, plant populations also contain evidence of their history. Because of this, and because man feels a duty to pass on items of heritage value to his successors (for their intrinsic value or because they may have potential for future use) there are sound philosophical grounds for retaining as much genetic diversity as possible within species and populations.

Plant populations and the genetic structures of those populations are not static, fixed entities: they are dynamic. The processes of gene flow, isolation (and the consequent possibilities of inbreeding depression), genetic drift, selection and mutation are continuously operating and will operate to modify the amount of biodiversity found, whatever management options are exercised. In the long term it does therefore seem unreasonable to expect to be able to maintain the full range of combinations of genetic variation currently exhibited in diverse and isolated populations. Nevertheless the concern shown to maximise the possibilities of doing so is understandable.

Among animals there are several well known cases where the distinct identity of a particular species has been thrown into doubt, or has been lost following the breakdown of reproductive barriers, usually following the invasion or introduction of more common species into areas formerly occupied by their rarer relatives. Examples include that of the extensive hybridisation

found to occur between mallard (*Anas platyrhnynchos*) and a number of rarer endemic duck species in various parts of the world (Rhymer & Simberloff, 1996). Similar hybridisation and introgression occurs in plants and may threaten rare, or endemic, species (Ouborg & Van Treuren, 1994). Introgression from domesticated forms of crop plants have also been implicated (directly or indirectly) in the extinction of wild populations of maize and rice (Gregorius & Steiner, 1993). The emphasis in all these cases has been on the loss of identity at the species or sub-species level because the rarities have been subsumed into their more common relatives. This is why there is a fear of genetic pollution.

Extended to an agricultural context the fear is that hybridisation may occur following the introduction of an agricultural crop into the vicinity of a population of a wild relative, so that some of the fitness genes selected as part of breeding programmes (particularly those which enable plants to establish sooner, grow more quickly or attain greater stature) may be transferred into wild populations, giving the hybrids such a competitive advantage that it may lead to their eventual extinction of the native species. Alternatively, the transferred genes may become dispersed throughout the whole population to produce a continuum between crop and wild species and, hence, loss of identity of the wild species. The development of transgenes, especially those conferring pest or disease resistance, is seen as cranking up this process, resulting in widespread modification of the genetic structure of wild populations and elimination of the variation they contain.

Gene movement between different species, or even genera, is not an unusual phenomenon as shown by the high proportion of hybrids present in the British and other floras (Stace, 1975; Ellstrand *et al*., 1996) but this general observation hides wide discrepancies in the frequency of hybridisation within different groups of plants. Among native British species, hybridisation is especially common in some families or genera, but rare in others (Table 1).

Table 1. Frequency of hybridisation in 7 genera of British plants (data from Stace, 1975)

Genus	Number of species	Number of interspecific hybrids	Number of intergeneric hybrids
Salix	23	123	0
Rumex (subgenus Rumex)	23	42	0
Geranium	17	7	0
Plantago	5	2	0
Dactylorhiza	7	27	22
Trifolium	21	2	0
Lolium (native species)	3	3	10

A contrasting situation is found in two genera which have a long history of agricultural use during which considerable infra-specific variation has been exploited or developed. Whilst there are strong breeding barriers between *Trifolium* species, *Lolium* species not only produce hybrids with each of their two congenors but are also capable of hybridising with *Festuca*, *Glyceria* and *Bromus* species. As the crop base in Britain (in terms of the original progenitor species) is relatively small, it is not surprising to find that even in crops with a long history of selective breeding and adaptation, the possibility of gene flow between them and wild relatives has not been eliminated. An increasing number of studies have shown specific cases where genes have passed to wild relatives from different crops and the whole topic has been reviewed by a number of authors, including De Vries *et al.*, (1992), Raybould & Gray (1993) and Gregorius & Steiner (1993). Assessments of relatedness may help determine the possibility of gene flow but it will not necessarily assist in predicting its actual incidence because gene flow rates between members of a crop-weed-wild plant complex may vary widely (Van Raamsdonk & Van der Maesen, 1996). Individual assessment on a species by species basis is necessary.

FACTORS INFLUENCING THE EFFECTIVENESS OF GENETIC POLLUTION

The process of gene flow has been discussed by many authors and extensively reviewed by Levin & Kerster (1967). All emphasise that a number of reproductive barriers have to be overcome before genes can be transferred successfully into new populations: barriers to dispersal, to compatibility and to post fertilisation processes. Gene flow from a crop to a relative, with which it can hybridise, is most likely to be accomplished directly by pollen dispersal because successful establishment following seed movement will depend on overcoming additional barriers to germination, establishment survival, flowering and seed set in an environment to which it may not be adapted.. Whichever route gene movement takes, provided the reproductive barriers are not insurmountable, a gene (or a transgene) may eventually be expected to become established in a close relative, even if dispersal rates are low.

As pointed out by Gregorius & Steiner (1993), once the transfer has taken place, subsequent consequences will be independent of the degree of relatedness between the crop and its wild relative. What will be more important are any effects that a transferred gene or gene complex may have in modifying fitness characteristics, and whether it does so independently of other fitness genes. Phenotypic plasticity may be able to compensate for some differences between growing conditions which exert selection pressure but, in crop plants artificial selection is designed to increase conformity to desired end points within a fixed environment, which reduces both genetic variation and plasticity, and so reduces the capacity for adaptation to non-agricultural conditions. In this respect crops differ from wildflower seed mixes, where the risk of "genetic pollution" is seen as being particularly high. There an intimate mixture of different species, each containing a range of genotypes is sown. Selection can act upon this variation, favouring those genotypes with more appropriate fitness traits at the expense of others. When plants containing adaptive genes developed to enhance agricultural production are transferred to situations where different selective pressures operate, the possession of those genes will not necessarily confer any selective advantage. Enhanced fitness of a weed/crop hybrid of radish (*Raphanus sativus*) has been demonstrated (Klinger & Ellstrand, 1994) but this was within an agricultural context and the results do not necessarily demonstrate a wider fitness capability in other environments. Whitton *et al.*, 1997 stress that the greatest opportunities for the

movement of transgenes are within the crop-weed complex and factors which enhance fitness in weedy populations, such as increased invasive capacity, are likely to me the most significant.

Where on the genome a gene occurs will influence the chances of its transfer from a crop because different parts of the genome may introgress at different rates. Such differential transfer has been shown to occur in crosses between *Brassica napus* and *B. campestris/rapa*, and in subsequent backcrosses (Mikkelsen *et al.*, 1996). It will also be influenced by whether the transgene in *B. napus* is on the A genome (derived from *B.rapa*) or the C genome (from *B. oleracea*). Once it is incorporated into a wild relative a gene closely associated with some other strong selectively-important gene or group of genes may also have a greater capacity for being retained and, ultimately, it could form part of what may become a highly conserved gene complex. Alternatively, if it behaves as a neutral gene, it will persist as a random component of the population gene pool, provided that mating within that population is also random. Raybould & Gray (1994) concluded that, indeed, many transgenes coding for proteins of no selective advantage in the wild (e.g. those conferring herbicide or antibiotic resistance, or used to produce pharmaceutical or industrial chemicals) will act as neutral genes. As there is no selective advantage, there will be no change in fitness.

THE HISTORICAL CONTEXT

Man has had a profound effect on evolutionary processes (selection, migration, drift, recombination, hybridisation and mutation) in many plant species since pre-historic times (Baker, 1972; Ledig, 1992). Indeed Brown (1992) goes so far as to claim that it would be difficult to find a species that has not been subject to some form of human-influenced selection. The value of certain plant species to man led him to impose his own selection criteria, initially collecting his crop and his seed from those individuals which were most suitable for his needs. Where this process occurred within natural populations, e.g. forests, there is evidence of considerable shifts in genetic structure depending on the harvesting technique used (Ledig, 1992). In primitive agricultural systems where the distinction between natural communities and planted areas was not clearly defined, and where wild and cultivated individuals remained essentially the same, opportunities for gene flow remained high. As a consequence Renno *et al.* (1997) assert that wild and cultivated forms of pearl millet (*Pennisetum glaucum*) have exchanged genes for the last 3000 years and have co-evolved over large parts of the Sahel.

In the course of agricultural development, some crop plants became increasingly differentiated from their wild relatives through domestication, defined by Van Raamsdonk (1995) as, "a process resulting in characteristics profitable for man [but] which generally reduces the fitness of plants in natural habitats." Reduction in fitness may be equated with loss of genetic diversity such that crop plants become increasingly confined to open environments where continuous disturbance is maintained by man. Through selection, plants are optimised for uniformity under these controlled conditions, whilst competition, diseases and pests are checked by a combination of selection, the use of effective agronomic procedures and the application of chemical agents. Where man has moved agricultural plants to new locations he has often narrowed the genetic base further by reducing the number of species used and their intra-specific diversity (Barrett, 1981). He has also taken his weeds with him. At the same time, if the introduced species have encountered novel ecological conditions, these may have been instrumental in changing life history and demographic characteristics (Eckert *et al.*, 1996).

Such processes might be expected to reduce the effective rate of gene flow between crop plants and their wild relatives. However, by breaking down geographical or ecological boundaries, new opportunities for hybridisation may be presented.

As Thirsk (1997) emphasised, farmers have often been extremely innovative in responding to difficult market conditions and opportunities for gene flow from new crops may well have arisen from the adaptation of cropping patterns which resulted from this innovation. Given their geographical proximity, and similar soils and climate, new crops with wild relatives already present in Britain, were most likely to have spread, in the Middle Ages, from the Low Countries and northern France to East Anglia and southern England. Thus, there is documentary evidence of rape (*Brassica napus*) being grown on the monastic estates of east Norfolk in 1255. Although vetches (*Vicia* spp.) were introduced before farm records were kept, evidence from the Bishop of Winchester's estate and from manorial records of the late thirteenth and early fourteenth centuries record the spread, with a distance decay pattern, of cultivated vetches from Kent along the coast to Hampshire and thence, via the chalk, to Berkshire, the Chilterns and, eventually, Norfolk. Clover (*Trifolium* spp.), lucerne (*Medicago sativa*) and sainfoin (*Onobrychis viciifolia*) were first recorded as a substitute for grass in the 1620s, although indigenous forms of clover may have been consciously used as fodder long before. The most widely-practised response to depressed grain prices in the early nineteenth century was to convert arable fields to pasture and, in doing this, farmers were urged not to use promiscuous grass seeds collected from a hay crop, even where it had come from a high quality field. The imported substitutes, sown under guidance from seed merchants' publications, which became an increasingly important source of advice, would have contained not only genetically different varieties, but also pernicious weeds (Sheail, 1986). Thus, over many centuries crop and weed species in the agricultural landscape must frequently have been exposed to imported genotypes (Ambrosoli, 1997).

A significant effect of farming in the past has been reduction of variability in weed species (Warwick, 1991) either through gene movement from crop relatives (though, of course, not all weeds are closely related to crop species and would not be subject to such gene flow) or the imposition of a strong selection agent. For example, reduction in polymorphism has been linked to increase in triazine resistance (Warwick & Black, 1993), though the extent of such reduction may vary between species from small, e.g. *Brassica rapa* to very large e.g. *Chenopodium album*. If agricultural systems have already reduced genetic variability in crops and some of the associated weed species, as well as reducing the capacity for phenotypic plasticity, plants within any weed-crop complex may be expected to resemble crop plants in having a low capacity for permanent spread beyond the confines of the agricultural environment. Although the constant and widespread introduction of novel varieties of crop plants may increase variation it will not increase fitness beyond the limits of cultivation, nor lead to the formation of locally-adapted gene complexes of the type found in land races of crop species, and which make them especially susceptible to gene mixing (Renno *et al.*, 1997). Similar gene complexes may also be present in highly adapted local populations and rare species but the threat of breakdown of these complexes, leading to disruption of the adaptive mechanism and extinction of the populations containing them is likely to be minimal because of the strength of geographical, ecological, taxonomic and reproductive barriers between them and most crop species. A few close relatives of crops do not exhibit the classical features of weed species and may be components of more clearly defined natural or semi-natural

communities. These include, for example *Daucus carota*, *Beta maritima* and some forage varieties of wild grasses and legumes.

CONCLUSIONS

Because the closest relatives of many crop plants are species with weedy characteristics, invading open or disturbed ground (and hence usually in close physical proximity to crops), barriers to introgression formed by reproductive and ecological isolation are small. Much larger barriers will exist where the relationship is distant, the populations are remote, the communities containing them are stable and closed, ecological conditions are very different and selection operates strongly in favour of locally-adapted genotypes. Under these circumstances, there is much less chance of incorporation of crop genes or transgenes into the genome of such plants. These factors lead to a conclusion that, because the mechanisms controlling gene flow from transgenes into wild relatives are much the same as those for other genes, there will be no significantly enhanced effect of transgenes on the genetic constitution of most components of the native flora, including many of the rarest species. The risks will be largely confined to close relatives which already share, through taxonomic links and past association with crops, a common history which is likely to have included extensive gene flow. Whether to improve understanding of the potential for gene flow in the past, or to help explain its present day significance, more extensive, yet detailed, study of the historic record might well repay the effort. Within this context, any incorporation of transgenes into weed species may be seen as a continuation of the developmental history of the crop/weed relative association, and not as an entirely new phenomenon.

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