Potential for gene-flow from cultivated Irish grasses and cereals

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PROJECT OUTPUT TO DATE

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1. Summary

The importance of gene movement from cultivated plants has been highlighted in regard to minimising the movement of seed and/or pollen between GM and non-GM crops (i.e. gene flow). Although ryegrass covers in excess of 90% of Ireland's agricultural area, very little is known about gene flow from ryegrass populations from an Irish context. The goal of this project was to address this lack of data by measuring the degree of pollen-mediated gene-flow between two *Lolium* spp. in a field environment. Ryegrass (esp. *Lolium perenne*) was selected because as the dominant pasture grass it is critical for the livestock industry as well as being a current target for novel improvements. The results from this research indicate that the potential for pollen-mediated gene flow from perennial ryegrass decreases exponentially with increased distance from the pollen source, with hybridisation events recorded out at 192m. In parallel to this research, a separate study was conducted to assess the degree of genetic diversity within feral and wild *Lolium* spp across Ireland and also within the important crop weed *Avena fatua* ('wild oats'); thereby providing an insight into the degree of historic gene flow that has occurred within each species and in regard to the latter, identifying the potential for non-native *A. fatua* to colonise the Irish agri-environment.

2. Introduction

Forage and turf grasses are of major economic importance in temperate farming regions and consequently are the focus of significant genetic improvement efforts worldwide, in particular for the optimisation of disease resistance and growth and nutritional characteristics (principally in *Lolium*, *Poa*, *Festuca* and *Agrostis* species). Increasingly this effort involves the use of genetically modified (GM) species (Sprangenberg et al., 1998).

In Ireland the dominant forage grasses are perennial and Italian ryegrass (*Lolium perenne* L. and *L. multiflorum* L., respectively), and the advent of suitable GM cultivars for deployment in Irish agroecosystems presents significant gene flow and coexistence questions. Ryegrass pastures, meadows and silage fields cover some 92% of Ireland's agricultural land and form the basis for the dairy and meat industries and much of the rural economy (Meade and Mullins, 2005). A significant portion of this land is managed by extensive grazing, giving rise to species-rich grasslands of considerable ecological and conservation value.

Lolium species are a particular concern for pollen mediated gene flow because they are obligate outcrossers and readily form hybrids both with each other and with several *Festuca* species (Webb et al., 1996). Significant pollen flow from *Lolium perenne* plots has been recorded (Giddings et al., 1997) and developed into a landscape gene flow model (Giddings, 2000). While this latter model did not incorporate the measurement of actual gene flow patterns as evidenced by pollination/ hybridisation events, this more precise kind of data has been generated in a landscape-level experiment on gene flow from a herbicide tolerant *Agrostis stolonifera* cultivar plot in the United States (Watrud et al., 2004). All three studies point to the extensive potential for gene flow from GM cultivar plots to surrounding receptive populations.

Existing research on major crop species shows that the spread of pollen tends to follow a leptokurtic distribution, i.e., it is concentrated in the areas immediately adjacent to the source and diminishes exponentially with increased distance from the source. Recent reports have demonstrated the potential for pollen-mediated gene flow over long distances for *Lolium rigidum* (Busi et al., 2008) and *Agrostis stolonifera* L. (Watrud et al., 2004). However, as yet there has been no research from an Irish context into this kind of gene-flow in grasses, even though they are by far the most important crop in Ireland.

Avena fatua (wild oats) is a native Irish species and an increasing weed problem in Irish arable fields (O' Mahony, 2003). Like the common cultivated oat (*A. sativa*) it is hexaploid, and the two species are interfertile (Stace, 1984). Infestations appear to have worsened in recent years with many areas recording sudden invasions where previously populations were maintained at a low level. This pattern may have arisen as a result of a number of factors: (i) contamination of seedstock; (ii) increased use of hired combine-harvesters; and (iii) seed spillage in the transportation of straw and grain. While Irish seed standards mandate 0% contamination in seedstock, seed from overseas often carries no such guarantee, opening the way for foreign wild oats varieties to contaminate Irish fields where growers buy cheaper supplies of seed from non-Irish sources.

Considering the ubiquity of pollen flow from grass swards and with a view to building a longer-term gene-flow modelling approach for *L. perenne* and *L. multiflorum* in the Irish agri-environment (Flannery et al., 2005), a strategic goal of this project is to develop a model for measuring pollen-mediated gene flow in field situations using heritable molecular and morphological markers. In addition, a genetic

survey of *Avena fatua* (wild oats) was to be completed to facilitate the completion of future crop (*A. sativa*) -to-wild (*A. fatua*) gene flow studies.

The potential for gene flow between related plants can be measured in a number of ways using the presence of morphological, physiological or genetic traits in hybrid progeny that result from a successful pollen-mediated gene flow event. In the case of morphological and physiological traits, dominant alleles must be targeted in order to record expression while for genetic traits both dominant and recessive alleles can be surveyed (Ellstrand et al., 1999).

Morphological characteristics provide a quick and cost effective tool of analysing large numbers of samples in direct gene flow experiments and have been used in many gene flow studies, sometimes as the sole markers for estimating the rate of gene flow (Halsey et al., 2005). However, as morphological traits may be under polygenic control, and so liable to display incomplete dominance in a heritability study, microsatellite (SSR) markers can be used as an additional, more definitive, means for establishing paternity in F_1 progeny produced in field-based gene flow trials. Microsatellite markers are highly suited to direct measurement of gene flow (Desplanque et al., 1999), particularly where alleles of different sizes arise in closely related (but reproductively isolated) species.

Therefore to assist in the acquisition of Irish-specific gene flow knowledge, the objectives of this project include the development of a model of genetic diversity within and between current populations of *L. perenne* and *A. fatua* and completion of (i) a protocol for identifying hybridization events between *L. perenne* and *L. multiflorum* in the field using a heritable molecular marker (microsatellite locus H01H06) and two heritable morphological markers (shoot structure and inflorescence awns) and (ii) a full scale field trial assessing gene flow from a *L. multiflorum* pollen source population to a surrounding *L. perenne* pollen sink population.

3. Methods

3.1 Selection of plant varieties for field-based gene flow study

A number of *L. perenne* and *L. multiflorum* advanced cultivar varieties were requested from the Institute of Grassland and Ecology Research (IGER), Aberystwyth, Wales and screened as to their suitability for a field gene flow experiment. Of the sample set, *L. perenne* cv. Aberdart and *L. multiflorum* cv. Trajan shared a heading date (the last week of May) that was suitable for the crop cycle at the Teagasc Crop Research Centre, Oak Park, and so were selected for preliminary microsatellite analysis.

3.2 Genotyping and modelling

Trial evaluation of microsatellite loci and AFLP primer combinations (n = 3) was completed for *L. perenne*. Sixteen primer pairs for microsatellite loci in *L. perenne* were assessed for their ability to distinguish between a selection of *Lolium* species from Ireland and Europe, including samples from recently collected Irish material. The microsatellite locus covered by the primer H01 H06 possessed alleles that clearly distinguish *L. perenne* from *L. multiflorum*.

DNA extraction was completed as follows: leaf tissue from 10 samples each of Aberdart and Trajan was dried in silica gel and ground into a powder using a Qiagen Retsch mm300 mixer mill and genomic DNA was isolated from each sample using a Machery Nagle Plant DNA extraction kit as per the manufacturer's protocols. Polymerase Chain Reaction (PCR) amplification of microsatellite loci utilized a standard PCR gel mix from BIOTOOLS that comprised 2 µl of DNA (in 100ul of Mahery Nagel elution buffer), 1.5 µl of forward and reverse primer, 2 mM $MgCl_2$, 1 unit of Taq polymerase, 200µM of dNTPs, 1X buffer and 25µl of PCR grade H_20 with a total reaction volume of 50µl. Optimisation of PCR annealing temperatures for microsatellite analysis was achieved using a 50 – 60^oC 8-step gradient PCR run on an Eppendorf Gradient Thermocycler with product visualised on an ethidium bromide-stained 4% Pronadisa agarose TAE gel run at 65V for 180 minutes. Primers for loci showing the desired fixation patterns were fluorescently labelled and following PCR amplification product was run on an Applied Biosystems AB310 Genetic Analyser. Fragment peaks from each sample run were then uploaded to Genotyper™ (Applied Biosystems) for alignment and size determination. Six microsatellite loci were selected for the *Avena*-based analysis (Table 1)

Table 1: Microsatellite loci selected for *Avena* genetic diversity analysis

Name	Forward and Reverse Primer Sequences	Repeat Motif	Size (bp)	Tm
AM3	ctg gtc atc ctc gcc gtt ca cat tta gcc agg ttg cca ggt c	(AG)35	280	51
AM4	ggt aag gtt tcg aag agc aaa g ggg cta tat cca tcc ctc ac	(AG)34	166	48
AM6	aat gaa gaa acg ggt gag gaa gtg cca gcc cag tag tta gcc cat ct	(AG)20	209	52
AM22	att gta ttt gta gcc cca gtt c aag agc gac cca gtt gta tg	(AC)22	138	46
AM30	tga aga tag cca tga gga ac gtg caa att gag ttt cac g	(GAA)14	203	43
AM31	gca aag gcc ata tgg tga gaa cat agg ttt gcc att cgt ggt	(GAA)23	186	47

A subset of Lolium samples were selected from the germplasm collection for analysis using three AFLP primer combinations (*Eco*RI –ACG/ *Mse*I –CAA; *Eco*RI –AGC/ *Mse*I –CAT; *Eco*RI –ACT/ *Mse*I –CTG). Raw fragment data generated on an AB310 genetic analyzer was collated using Genescan 3.1 and transferred to Genotyper 2.5 where all samples were aligned and fragment peaks translated into exact base-pair sizes. This data was transferred to an Excel spreadsheet and converted into a presence/absence binary code matrix. The matrix was (i) converted to nexus file format and entered into PAUP 4.0 for cluster analysis and (ii) entered into GENALEX 6.0 for multivariate analysis using Principal Coordinates analysis (PCO) (two methods for estimating genetic relatedness between samples).

3.3 Selection of discrete morphological traits

An examination of Hubbard's taxonomic descriptions of *Lolium* species that occur in Ireland suggested the most useful taxonomic characters for differentiating between *L. multiflorum* and *L. perenne* are the presence of awned inflorescences and rolled shoots in *L. multiflorum*, versus inflorescences without awns and shoots that are folded just once in *L. perenne* (Hubbard, 1984), as illustrated in Figure 3.

A survey of 50 plants each from the IGER *L. multiflorum* cv. Trajan and *L. perenne* cv. Aberdart seedlots revealed that these characters were both discrete and consistent for identifying plants to species. In addition, preliminary field surveys in 2001 across Ireland suggested that awns are frequently displayed by hybrids where perennial and Italian ryegrasses co-occur. Perennial ryegrass does also differ from Italian ryegrass in its greater longevity (*L. multiflorum* is typically an annual plant), however in a short-term experimental scenario the use of morphological presence/ absence traits provides a more time-efficient means for visual spot-checking for hybrid progeny between the two species.

3.4 Field hybridisation study

A site was established in Oak Park with 500 pollen source plants (*L. multiflorum* Trajan) surrounded by eight concentric rings of sink plants (*L. perenne* Aberdart), ranging 8 to 192m in radius and containing c. 500 plants in total. Rings 128, 160 and 192 were incomplete. The sink plants (illustrated in Figure 2) were arranged exactly 6.28m apart in order to standardize the local pollen flow environment between neighbouring sink plants across all rings. Each plant was protected from grazing rabbits/hares with chicken wire. Over the course of April to August all of the trial plants were allowed to mature and flower. Potential hybrid seed was gathered on a weekly basis thorugh the first three weeks of August, c. 8 weeks after initial heading that had been delayed by poor weather.

3.5 *Lolium h*ybrid analysis

Putative hybrid seed was sown in compartmentalised trays (12 seeds / tray) under glasshouse conditions to maximise germination and facilitate hybrid analysis. For week 1, 302 trays were used, while 378 trays were used in week 2 and 276 trays for week 3. Hybrids were first identified amongst the F_1 progeny by spot checking for the presence of *L. multiflorum* morphological traits (awned inflorescences and a rolled basal leaf shoot in Trans Section). Each plant was then analysed for the presence of *L. perenne* and *L. multiflorum* SSR specific-alleles.

3.6 Sampling and creation of *Avena* germplasm collection

Sampling of wild oats (*A. fatua*) was conducted during August/September with seed collected from 10 commercial/fallow fields (Table 2). In addition, *Avena* samples were received from the Institute for Grassland and Ecology Research (IGER), Aberystwyth, Wales; the Millennium Seed Bank, Royal Botanic Gardens Kew, England, and the Institute for Plant Genetics at Gatersleben, Germany. All germplasm was stored at –20^{o}C. DNA was extracted as above for SSR/AFLP-based genotyping.

Table 2: *A. fatua* samples used for microsatellite analysis to determine levels of genetic diversity in national populations

Sample	Site ID	Number of samples
Oak Park, Co. Carlow (site 1)	A1	12
Oak Park, Co. Carlow (site 2)	A2	12
Rathkeale, Co. Limerick.	A3	10
Inishannon, Bandon, Co. Cork (field 1)	A4	10
Inishannon, Bandon, Co. Cork (field 2)	A5	10
Villierstown, Co.Waterford	A6	10
New Ross, Co. Wexford	A7	7
New Ross, Co. Wexford	A8	3
Lyons Estate, Co. Dublin	A9	12
Bellewstown, Co. Louth	B	10
Overseas		
Poland		1
Slovakia		1
Related Outgroup Taxa		
Avena strigosa		1

4. Results

4.1 Modelling genetic diversity within and between current populations of *A. fatua* and *Lolium perenne*

Following the generation of a germplasm collection for both *L. perenne* and *A. fatua*, the goal of this sub-project was to assess the degree of genetic diversity and levels of gene-flow within each species. Evaluation of microsatellite loci and AFLP primer combinations was completed for *L. perenne*, with primers for microsatellite loci in *L. perenne* assessed for their ability to distinguish between a selection of *Lolium* species from Ireland and Europe, including samples from recently collected Irish material. Six microsatellite loci (AM3, AM4, AM6, AM22, AM30, AM31, Table 1) were selected to identify genetic variation and gene-flow in *Avena* species.

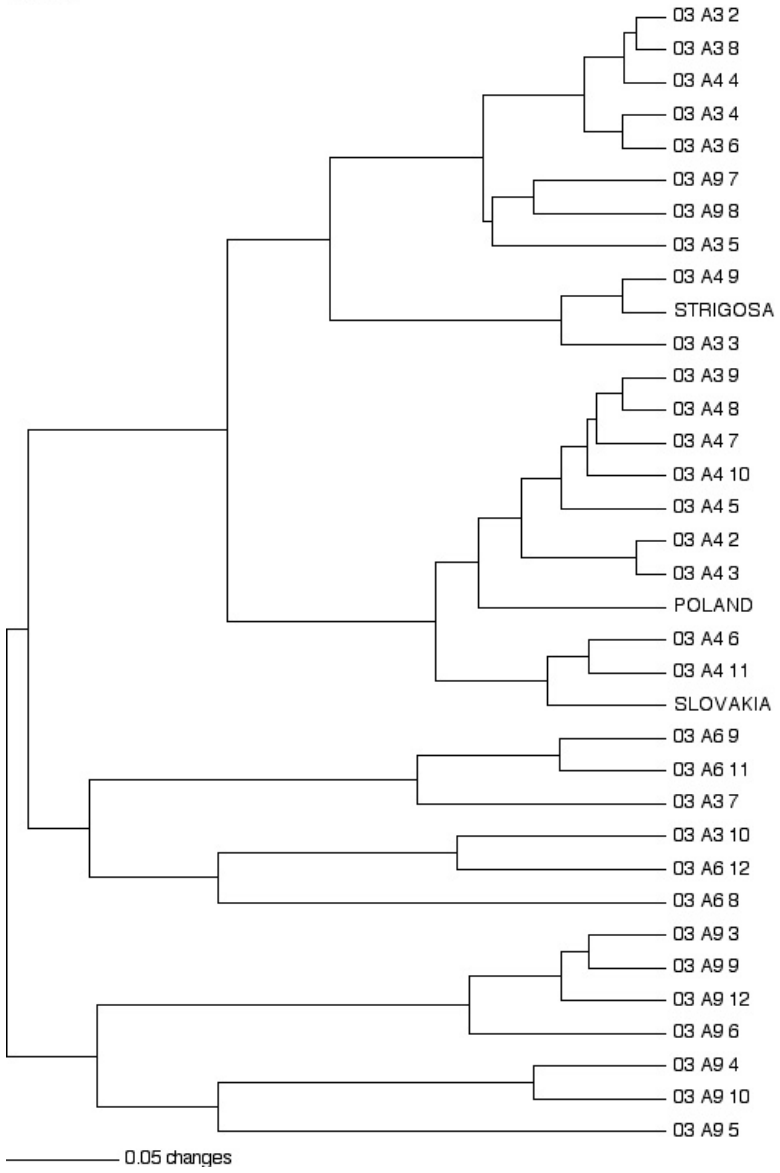
The microsatellite analyses revealed a number of genetic patterns within the sampled Irish *A. fatua* populations. Mean levels of heterozygosity and the total number of private bands per population varied greatly. For example, population A9 (Lyons Estate, Co. Dublin) appears to contain a slightly higher overall level of genetic diversity than A6 (Villierstown, Co. Waterford), with a higher number of private bands recorded at both loci, and a slightly higher mean heterozygosity for both loci also, even though the sample size at AM30 for A9 is much larger than for A6. As such the method used in this project appears to be consistently sensitive to detect small differences between populations.

Larger differences are also evident from the dataset. For example, populations A4 and A3 analysed with locus AM30 are almost equivalent in size (10 alleles as against 9), however for A4, 7 of the 10 recorded bands are private and shared with no other population, compared to just 1 of 10 for A3. A4 also shows a much higher level of mean heterozygosity at 51.61% compared to 32.26% for A3. Crucially, two of the populations contiguous with A4 are the *A. fatua* samples from Poland and the Czech Republic. Population genetics theory developed from the Hardy-Weinberg Equilibrium (HWE) model suggests that samples drawn from large populations should have greater genetic diversity compared to those drawn from smaller populations (Hartl, 2000).

As such, if we are to identify recent foreign-origin weed infestations in wild oats then it is likely they will be both divergent from Irish populations and also more genetically diverse than the average Irish sample. This is exactly the scenario recorded here with population A4, which is divergent from other Irish populations and also has close affinity with the European samples in the UPGMA diagram in Figure 1. We can thus conclude that amongst all the populations sampled in this study, A4 is the most likely to be of recent foreign origin.

In addition to this finding, many Irish samples aggregate about a common genetic identity in a separate principle component analysis, providing some support that there is a ‘typical’ Irish genotype for *A. fatua*, most evident perhaps in the character of populations A6 and A9.

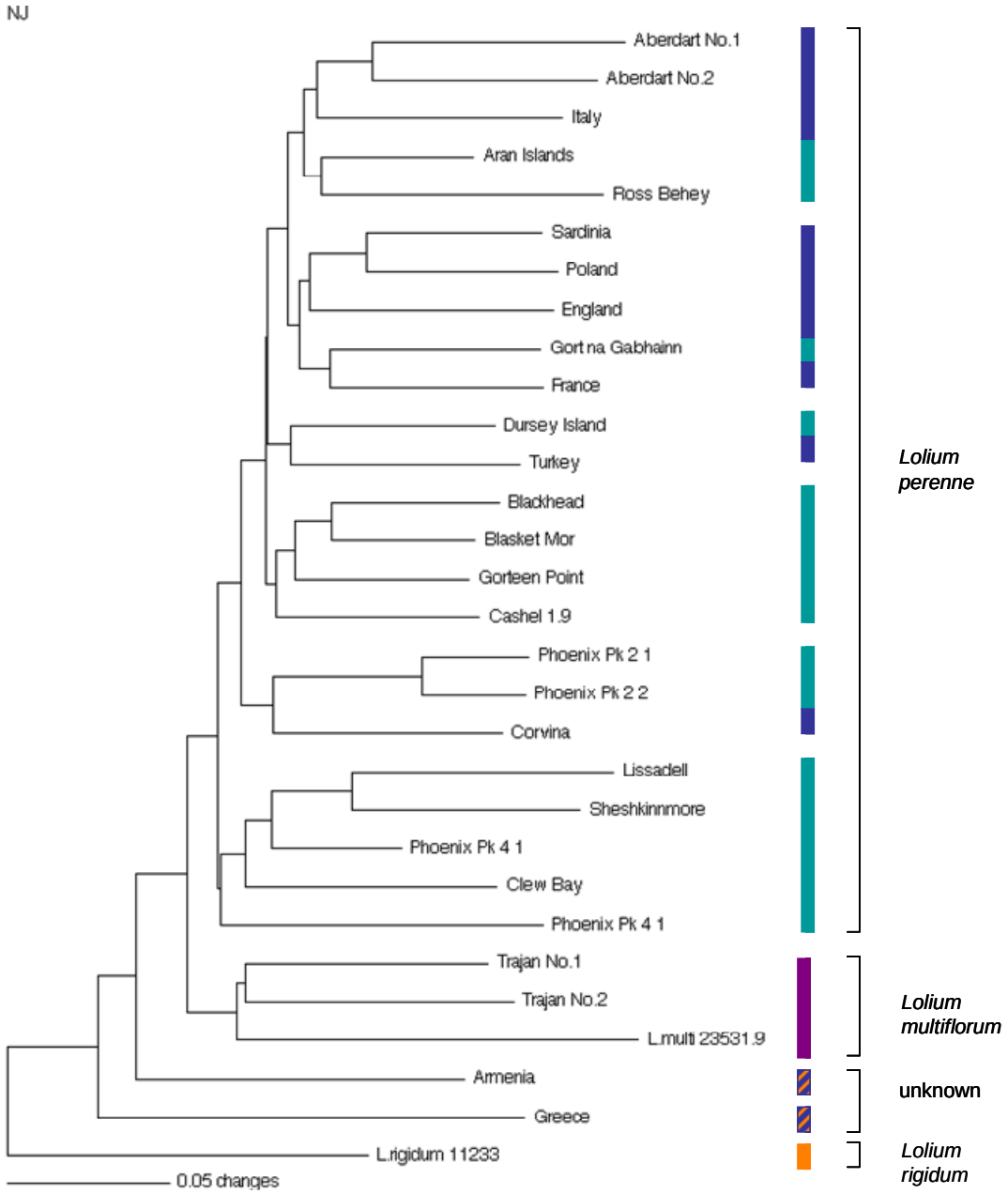
Fig.1. Hierarchical cluster diagram of individual *A. fatua* samples generated from microsatellite locus AM30. Binary data using the UPGMA algorithm on PAUP 4.0.



For the *Lolium* spp., the genetic relationship tree clearly separates *L. rigidum* and *L. multiflorum* from the *L. perenne* samples and within this latter group there is a suggestion that the Irish samples share a complex relationship with the wider European population (Figure 2). The analysis appears quite sensitive to differences and similarities in local Irish populations: two sites at the Phoenix Park (2 and 4) are separated on the tree while sites at Gort na Gabhainn (upland unimproved pasture) and Ross Behy (sand dune commonage), just 10 km apart on the Iveragh Peninsula in Co. Kerry, are also separated on the tree. Equally, while Blackhead and Gorteen point

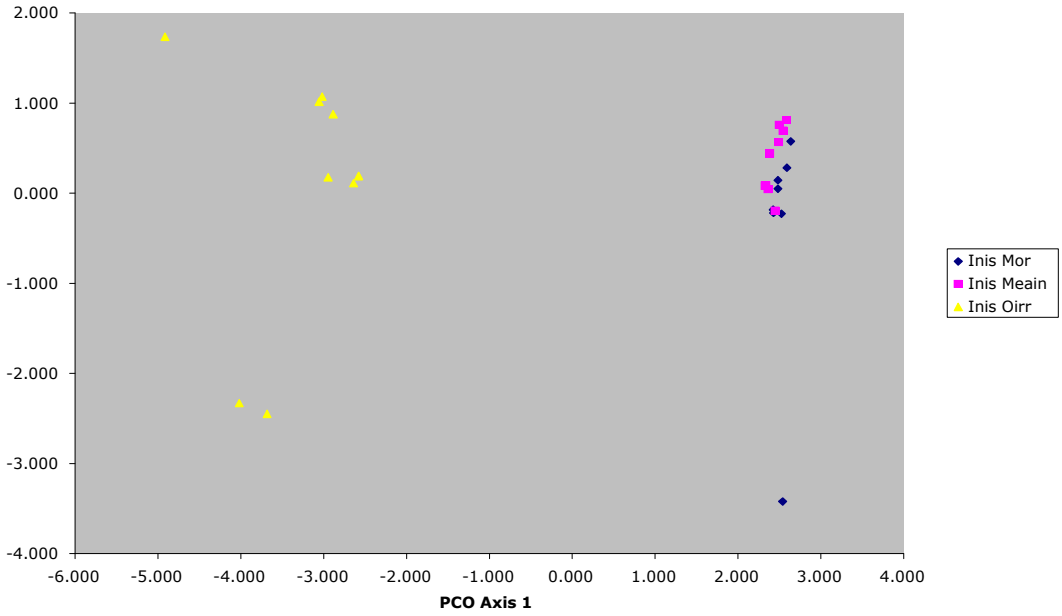
(on the southern and northern shores of Galway Bay) appear to be closely related, the genotype from Inis Oírr at the mouth of Galway Bay is quite distinct from both. The

Fig. 2. Genetic relationship tree for a trial selection of *Lolium* samples based on fragment data generated using 3 AFLP primer sets described in Table 2.4 and analysed using the Neighbor Joining clustering algorithm in *PAUP 4.0*. *Lolium rigidum* (at the base of the tree) is the outgroup, or most divergent sample. *Lolium multiflorum*, including variety Trajan used in the gene-flow experiments, is the next most divergent, while all samples above the *multiflorum* branch are *L. perenne*. Irish and European samplee of *L. perenne* are indicated by green and blue shading, respectively.



two samples from the Northwest at Lissadell Co. Sligo and Sheshkinmore, Co. Donegal appear closely related. Ross Behey, Gort na Gabhainn and Inis Oírr are all located within a large cluster of European samples. Examining *Lolium* populations contained within a defined geographic area (e.g. Aran Islands – an enclosed and traditional grassland system with limited contamination by new/ foreign ryegrass cultivars), it was clear that the populations on Inis Meain and Inis Mor share a close genetic affinity that is somewhat removed from those on Inis Oirr (Figure 3).

Fig.3. Principal Coordinates Analysis of microsatellite data generated from the loci HO1AO2, HO1FO2 and HO1HO6

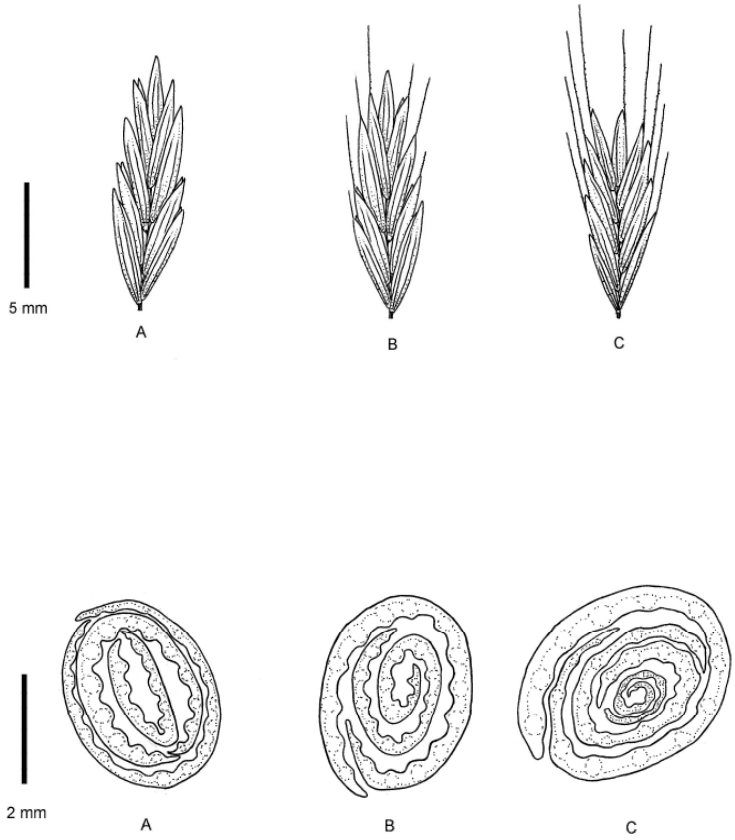


4.2 Developing protocols to identify hybridization events between *L. multiflorum* and *L. perenne* in the field

The objective of this task was to develop both molecular and morphological markers to assist in quantifying the rate of pollen-mediated gene flow between *L. perenne* and *L. multiflorum* in a field environment. This was achieved using a heritable molecular marker (microsatellite locus H01H06) and two heritable morphological markers (shoot structure and inflorescence awns).

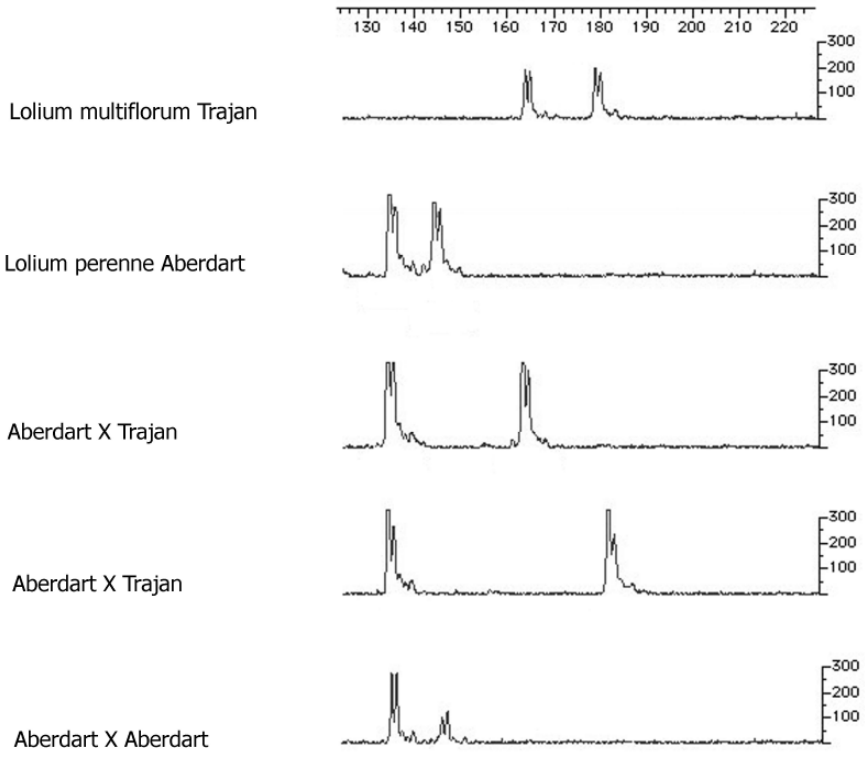
The screening of morphological traits in *L. perenne* Aberdart and *L. multiflorum* Trajan identified two characters (occuring in Trajan but not in Aberdart) that could potentially identify hybrid seed progeny from Aberdart sink plants in the field trial: (i) the presence of awns on the inflorescence and (ii) the presence of a coiled shoot in juvenile plants. The presence versus absence of awns in the inflorescence (Figure 4) proved to be a more reliable marker of *L. multiflorum* paternity in *L. perenne* maternal progeny than the rolled versus folded shoot habit.

Fig.4. (A) Inheritance of inflorescence characteristics in *L. perenne* X *multiflorum* hybrid crosses (A) Inflorescence structure of *Lolium perenne*. (B) Inflorescence structure of hybrid *L. perenne* X *multiflorum* showing inflorescence with awns inherited from the paternal *L. multiflorum* parent. (C) Inflorescence structure of *L. multiflorum*, showing the presence of awns on the lemmas and fruits. **(B)** Inheritance of shoot characteristics in *L. perenne* X *multiflorum* hybrid crosses. (A) Trans section through shoot of *Lolium perenne*, showing folded leaf blades. (B) Trans section through shoot of *L. perenne* X *multiflorum* hybrid, showing rolled leaf blades inherited from paternal *L. multiflorum* parent. (C) Trans section through shoot of *L. multiflorum*, showing rolled leaf blades.



The screening of 16 sampled *Lolium*-specific microsatellite loci showed that several loci displayed polymorphism across the initial sample set of 10 *L. multiflorum* Trajan and 10 *L. perenne* Aberdart plants, however fixation of species-specific alleles was clear only at locus H01H06, with a clear size difference between the alleles evident in Trajan compared to Aberdart (Figure 5). As the microsatellite alleles from both parents are detectable, this is a more reliable method than morphological traits for the purposes of identifying paternity (as morphological traits of the paternal parent can be recessive and so go undetected in hybrid progeny)

Fig.5. Microsatellite allele profiles in *L. multiflorum* Trajan, *L. perenne* Aberdart and hybrids between these two species as recorded at the HO1 HO6 locus. Horizontal scale indicates the size of fragments in nucleotide base pairs (bp). Vertical scale indicates fluorescence level of fragments, a general indicator of fragment quantity.



4.3 Complete a full scale field trial assessing gene flow from a *L. multiflorum* pollen source population to a surrounding *L. perenne* pollen sink population

Following the development of methods for monitoring gene-flow from source plants (*L. multiflorum* Trajan) to sink plants (*L. perenne* Aberdart) a full scale field experiment was carried out at Oak Park, in Carlow. The layout of Lolium plants (Figure 5) was designed so that the pollen source Trajan plants were located on an elevated platform at the centre of the plot with the sink Aberdart plants located in concentric circles (in an existing crop of sugar beet) that extended to the margin of the plot. Both Aberdart and Trajan sample plants flowered at the end of June.

Approximately 60% of Trajan plants flowered in week one, a further 20% in week two and 10% in week 3. For each of these three weeks all Aberdart inflorescences that started flowering were labeled week 1, week 2 or week 3. As 90% of the Trajan source plot plants had finished flowering by the end of week 3 labeling of Aberdart plants was ended. Mature seed was collected from each Aberdart inflorescence 4 weeks after it first flowered. Each sink plant therefore produced three sets of harvested seed, corresponding with the flowering period, which underwent

paternity analysis using a combination of the awned inflorescence scoring method and microsatellite analysis.

Fig.6 A. Layout of gene-flow field experiment at Oak Park. The plot of 500 pollen source plants (*L. multiflorum* Trajan) was surrounded by eight concentric rings of sink plants (*L. perenne* Aberdart), ranging 8 to 192m in radius and containing c. 500 plants in total. Rings 128, 160 and 192 were incomplete. The sink plants were arranged exactly 6.28m apart [= ***d***, cirumference/radius = 6.28] in order to standardize the local pollen flow environment between neighbouring sink plants across all rings.

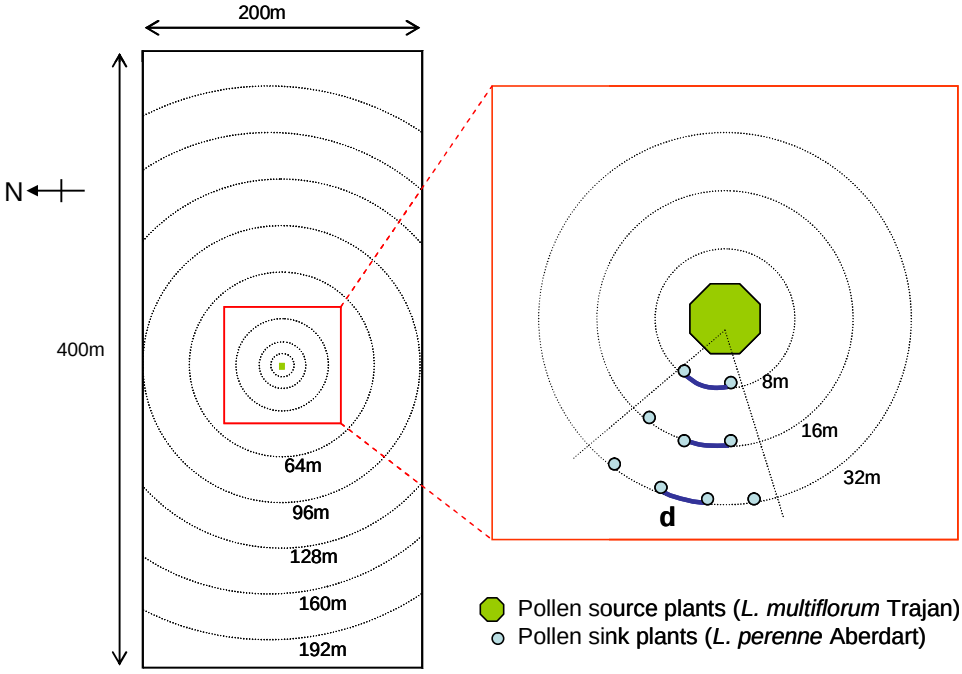
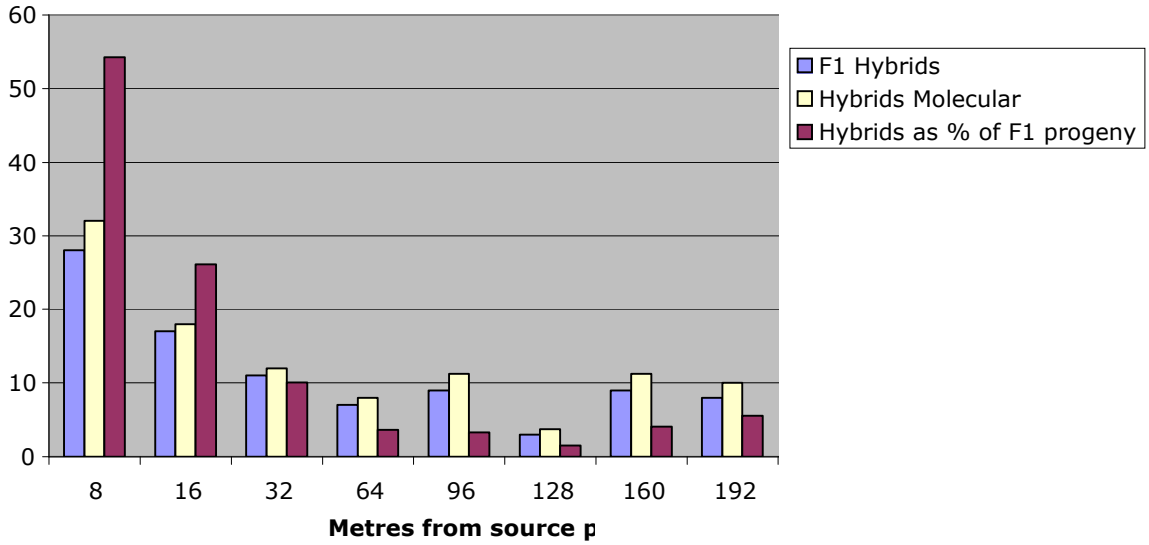


Fig.6 B. Representative individual pollen sink plant (*L. perenne* Aberdart).

Significantly, hybrids were found at the plot perimeter, 192m from the central pollen source plot (Figure 7). An approximate inverse exponential gene flow curve is evident in the dataset which is in line with existing gene flow studies. This tendency will point towards a complex long range gene flow model governed by random/ stochastic gene flow events in the wider landscape.

Fig.7. Total number of hybrids in pollen sink F_1 progeny as scored by morphological marker (blue), by molecular marker (yellow) and as % of F_1 generation at concentric distance that score positively for molecular markers (red)



A time-series analysis for hybrid occurrence up to 64m from the source plot over three successive weeks of the experiment confirmed that the onset of flowering in both the source and sink plant populations resulted in peak pollen flow for a period of a single week. This was followed by a rapid drop-off of pollen flow over the following two weeks.

Combined with the distance data, the presented composite model shows pollen flow to have an inverse exponential distribution in space and time: an empricial demonstration of what has been suggested in theory and by inference from other work. It is clear however that beyond 64m from the pollen source, the gene flow curve becomes stochastic. This result has significant implications for the introduction of an efficient management regime to ensure the successful coexistence of GM and non-GM varieties of ryegrass as it would be difficult to determine a minimum isolation distance to ensure potential admixture of GM into non-GM swards does not exceed the mandated 0.9% threshold.

5.0 Conclusions

Agricultural biotechnology ('ag-biotech') is becoming increasingly important for the viability and sustained competitiveness of agriculture both in Ireland and worldwide. One application of ag-biotech is the generation of GM crops, whose successful deployment is dependent upon the generation of adequate knowledge of their behaviour in the field. Gene-flow, via pollen spread, from GM crops to wild and cultivated relatives is one of the most pressing environmental issues associated with the technology.

Employing tools of biotechnology (microsatellite analysis), this project has quantified the degree of pollen-mediated gene flow from perennial ryegrass populations in the field. Utilizing the microsatellite locus H01H06, the degree of hybridisation between *L. perenne* cv. Aberdart x *L. multiflorum* cv. Trajan was successfully traced. The number of hybrids identified using morphological traits differed from the number identified using the microsatellite marker. In the case of the elongated awns, the number of hybrids recorded was lower than for the microsatellite total, and each plant that carried awns also scored positively for the *L. multiflorum* microsatellite marker. The rolled leaf shoots character proved more unreliable, recording more putative hybrids than the microsatellite marker in all three plots. Significantly several of the plants showing what appeared to be a rolled shoot did not score positively for the *L. multiflorum* microsatellite marker, while several others that did score positively for this microsatellite marker did not show the rolled shoot habit. In terms of larger scale experiments, because it is not feasible to analyse all F_1 progeny using microsatellite-based analysis (the total F_1 seed in a large trial may number 10 or 20,000) it would appear that hybrid progeny can be scored using the elongated awns character (but not the rolled shoot character), provided a more accurate estimate of under-recording of hybrids can be generated.

From the research conducted in this project it is evident that hybridisation rates in a large grass gene-flow field experiment can be measured using a combination of the listed cultivars and the H01H06 marker, and that interspecific hybridisation between *L. multiflorum* pollen donors and *L. perenne* pollen receptors is possible even in the presence of pollen competition from adjacent *L. perenne* plants; which inhabited hedgerows adjacent to the site of study. In terms of the broader GMO biosafety research effort, these results provide further evidence that field assessment of gene flow patterns within and between wind-pollinated species need not require the deployment of GM varieties for the measurement of actual gene flow patterns.

The genetic diversity analyses of *Lolium* spp. did succeed in differentiating between *L. perenne* and *L. multiflorum* / *L. rigidum*. The analysis also identified a possible subgroup of Irish *L. perenne* comprising the Phoenix park meadows and samples from the northwest and it appears that most Irish old meadows and pastures share overlapping genetic identities that in many cases have as close relationships to distant European populations as they do to local Irish populations. The inference from the isolated study conducted on the Aran Islands, is that there have been strong gene-flow relationships between *L. perenne* populations on Inish Meain and Inis Mor, whereas gene flow between these two islands and Inis Oirr has been much less frequent. This pattern may reflect the traditionally closer relationship between the islanders of Inis Oirr and coastal communities in Co. Clare around Doolin, which often involves the transfer of livestock to and from the mainland. These results confirm that population-level analyses are much more likely to yield meaningful gene-flow data than large-scale national assessments.

The wild oat (*Avena fatua* L.) is a native Irish species and a significant weed problem in Irish arable fields (Webb et al., 1996). Infestations appear to have worsened in recent years with many areas recording sudden invasions where previously populations were maintained at a low level. While Irish seed standards mandate 0% contamination in seedstock, seed from overseas often carries no such guarantee, opening the way for foreign wild oats varieties to contaminate Irish fields where growers buy cheaper supplies of seed from non-Irish sources. The goal of this project was to genetically characterise varieties of Irish wild oats and discriminate between these and new foreign populations that may have been introduced in recent years via contaminated seed supplies. The results demonstrate the utility of microsatellite markers for the purpose of identifying and characterizing different Irish populations of *A. fatua*. A majority of sampled Irish ecotypes aggregate about a common genetic identity, which provides support to the hypothesis that there is a 'typical' Irish genotype for *A. fatua*, most evident perhaps in the character of populations A6 and A9. In particular, the completed molecular analysis identified one population A4 (Inishannon, Co. Cork) which shows all the characteristics of being of recent foreign origin.

6.0 References

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