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ORIGINAL PAPER

Lack of genetic structure and evidence for long-distance dispersal in ash (*Fraxinus excelsior*) populations under threat from an emergent fungal pathogen: implications for restorative planting

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Abstract Genetic analysis on populations of European ash (*Fraxinus excelsior*) throughout Ireland was carried out to determine the levels and patterns of genetic diversity in naturally seeded trees in ash woodlands and hedgerows, with the aim of informing conservation and replanting strategies in the face of potential loss of trees as a result of ash dieback. Samples from 33 sites across Northern Ireland and three sites in the Republic of Ireland were genotyped for eight nuclear and ten chloroplast microsatellites. Levels of diversity were high (mean A_R = 10.53; mean H_O = 0.709; mean H_E = 0.765), and were similar to those in Great Britain and continental Europe, whilst levels of population genetic differentiation based on nuclear microsatellites were extremely low (Φ_{ST} = 0.0131). Levels of inbreeding (mean F_{IS} = 0.067) were significantly lower than those reported for populations from Great Britain. Fine-scale analysis of seed dispersal indicated potential for dispersal over hundreds of metres. Our results suggest that ash woodlands across Ireland could be treated as a single management unit, and thus native material from anywhere in Ireland could be used as a source for replanting. In addition, high potential for dispersal has implications for recolonization processes post-ash dieback (*Chalara fraxinea*) infection, and could aid in our assessment of the capacity of ash to shift its range in response to global climate change.

ADDITIONAL KEYWORDS**:** Gene flow, genetic diversity, inbreeding, microsatellites, spatial genetic structure, replanting

Introduction

In recent years, many ecologically and economically important tree species have come under threat from a range of emergent pathogens. The outbreaks of the fungus *Ophiostoma novo-ulmi*, the agent of Dutch elm disease in the 1900s, led to extensive losses of several *Ulmus* species, including an estimated two-thirds of the elm population of the UK during the 1970s (Webber 1981). In the last decade in the UK and Ireland, notable fungal and oomycete pathogens have included sudden oak death, chestnut blight and red needle blight. Most recently, outbreaks of ash dieback, a potentially serious threat which affects several species of ash (*Fraxinus* spp.), have been reported in continental Europe, and have subsequently spread to Great Britain and Ireland. Common or European ash (*F. excelsior*) is a key species of mixed broadleaved woodlands across Europe, with a natural range that extends from southern Scandinavia to northern Spain and the Balkans, and from Ireland in the west to continental Russia in the east. European ash within woodlands forms mixed stands, usually with beech (*Fagus sylvatica*), pedunculate oak (*Quercus robur*), sessile oak (*Q. petraea*), alder (*Alnus glutinosa*) and sycamore (*Acer pesudoplatanus*), and is an important component of woodland ecosystems, as well as being a valuable timber species (FRAXIGEN 2005). The symptoms of ash dieback were first reported in Poland in the early 1990s (Pautasso et al. 2013), but it was not until 2006 that the causative agent of ash dieback was identified as *Chalara fraxinea* (Kowalski 2006), which has since been found to be synonymous with the ascomycete fungus *Hymenoscyphus pseudoalbidus* (Queloz et al. 2011). The disease was first recorded in Britain in February 2012, and the first case of ash dieback in Ireland was reported in October 2012.

Replanting of forests will have to be considered if ash dieback outbreaks result in substantial loss of trees, either via pathogenic mortality or anthropogenic clearance to prevent

45 possible spread. In Great Britain, the Forestry Commission has developed recommendations
46 to maintain provenance of replanted individuals, by using seed sourced from the same area
47 (Herbert et al. 1999). Consequently, a map of "seed zones" that divide Great Britain into 24
48 areas delineated by geographic features and general climatic similarity has been drawn up to
49 assist restorative conservation programmes. However, a recent study on ash in England,
50 Scotland and Wales (Sutherland et al. 2010) found limited genetic differentiation between 42
51 populations from 21 of the 24 seed zones, indicating large-scale genetic homogeneity. This
52 suggests that all populations of ash in Britain could be treated as a single management unit
53 (DeSalle and Amato 2004), a more efficient and cost-effective approach to replanting,
54 contrary to recommendations based on previously identified "seed zones".
55 Seed dispersal plays a central role in the demography of natural plant populations across a
56 broad range of geographic scales, from initial colonization to shaping community structure
57 and regeneration (Howe and Smallwood 1982; Nathan and Muller-Landau 2000; Levine and
58 Murrell 2003). Despite the importance of dispersal in plant population ecology, the logistics
59 of tracing dispersal events accurately from source are not straightforward. Methods
60 involving "tagging" of seeds are generally less than optimal due to factors such as extremely
61 low recovery rates and the effects of the tags themselves on the dispersal process (reviewed
62 in Wang and Smith 2002; Forget and Wenny 2005; Ashley 2010). Most attempts to estimate
63 seed dispersal distributions have instead relied on seed trapping, coupled with models that
64 generally make *a priori* assumptions about seed source (Nathan and Muller-Landau 2000).
65 In recent years, climate change, habitat loss and fragmentation, and increased mortality
66 associated with emergent plant pathogens, such as *H. pseudoalbidus*, have increased interest
67 in more direct, precise measurements of seed dispersal to determine the capacity of plant
68 populations to recover from these threats.

With the recent report of the first case of ash dieback in Ireland, and the lack of population genetic information for the species across the island, the main aim of the present study was to determine the levels and patterns of genetic diversity in naturally seeded trees in ash woodlands and hedgerows. We focused on Northern Ireland which, like the rest of Ireland, has no map of "seed zones" on which to base management units, and the development of rational conservation and replanting strategies. We analysed populations from the northern, eastern, southern and western extremes of Ireland to ensure our findings are applicable to the island as a whole. We also used a molecular genetic approach to quantify fine-scale seed dispersal distances in two natural ash woodlands, employing a combination of high-resolution nuclear and chloroplast microsatellite markers. Our results suggest that ash woodlands across Ireland could be treated as a single management unit, and thus material from anywhere in Ireland could be used as a source for replanting. We also identified potential for seed dispersal over hundreds of metres, which will be important in addressing both post-ash dieback recolonization, and assessing the capacity of ash to migrate in response to global climate change.

Materials and methods

Study species reproductive ecology

European ash (*Fraxinus excelsior*) has protandrous, anemophilous flowers. The species exhibits a wide range of complex polygamy, ranging from pure male and female trees, through combinations of male / female and hermaphroditic flowers in the same individual, to sexual changes across successive years (Wardle 1961; Bacles and Ennos 2008). Although *F. excelsior* is preferentially outcrossing, hermaphrodites are self-compatible, and whereas females and hermaphrodites exhibit high seed set, hermaphrodites exhibit reduced male fertility. Fruits are winged and wind-dispersed, and generally contain a single seed. Seedlings are shade-tolerant, but need good light levels to promote full growth, generally only establishing in clearings within woodlands (Marigo et al. 2000).

Sampling and DNA extraction

For the broad-scale study, samples were collected from 33 sites across Northern Ireland and three sites in the Republic of Ireland that had been previously designated as ancient or semi-natural woodland based on data collected for the Woodland Trust Inventory of ancient and long-established woodland in Northern Ireland (www.backonthemap.org.uk) and the National Survey of Native Woodlands 2003-08 in the Republic of Ireland (www.npws.ie; Fig. 1 and Table 1). The congeneric *F. angustifolia* has been planted in the Republic of Ireland, but is not found in the vicinity of any of the native woodlands analyzed in the present study. A single leaf was collected from each of 30 trees per site and stored in silica gel, and GPS coordinates recorded for every tree sampled. DNA was extracted using the CTAB method of Doyle and Doyle (1987).

For the fine-scale study, two sites were chosen. The first, Barnett Demesne, was also used for the broad-scale study. It is a *ca*. 40 ha public park in South Belfast, Northern Ireland (54.55° N, 5.96° W – Fig. 2), and is an area of mixed parkland and woodland, the woodland being semi-continuous stands of mixed deciduous trees, primarily beech and oak. The ash is found in the northern part of the main wooded area, with a few scattered trees in the adjoining parkland. The second site, Cregagh Glen, is a narrow (50 – 60 m), steep-sided ravine *ca.* 700 m long on the eastern outskirts of Belfast (54.56° N, 5.89° W – Fig. 2). It is the surviving remnant of a former *ca*. 400 ha forest and comprises mixed woodland of Scots pine, sycamore, beech and ash. The ash is distributed sporadically throughout the length of the Glen. For both sites, samples were obtained from all reproductive (adult) trees, as well as from selected saplings (96 from Barnett Demesne and 48 from Cregagh Glen; Figure 2). A single leaf was collected from each individual and stored in silica gel, and GPS coordinates recorded (Table S1, Supporting Information). DNA was extracted using the CTAB method of Doyle and Doyle (1987).

Genotyping

All trees and saplings were genotyped for eight nuclear and ten chloroplast microsatellite loci. For nuclear microsatellite genotyping, we used six previously reported loci which have been widely used in population genetic studies on ash: Femsatl-4, Femsatl-8, Femsatl-11, Femsatl-16 and Femsatl-19 (Lefort et al. 1999) and M230 (Brachet et al. 1999), as well as two loci developed for the present study (FR639485 and FR646655). As previous studies highlighted the possibility of null alleles using the Lefort et al. (1999) and Brachet *et al.* (1999) primers (Morand et al. 2002; Ferrazzini et al. 2007; Sutherland et al. 2010), we designed new primers for all loci (Table 2) using the Primer3 program (v 0.4.0; http://primer3.ut.ee). The F_{IS} values calculated in the present broad-scale study were lower

than those from several previous studies, which is consistent with the occurrence of null alleles when using the original primers. To investigate this further, we also genotyped a subset of our samples for comparison using the original Femsatl-4, Femsatl-8 and Femsatl-16 primers, since these exhibited the highest F_{IS} values in the earlier studies. To develop further markers, we also tested five pairs of primers developed from EST sequences in GenBank, but only two of these (FR639485 and FR646655) consistently gave clear, reproducible products.

Fraxinus excelsior chloroplast sequences in the GenBank database were searched for mononucleotide repeats of ten or more (Provan et al. 2001). Primers were designed using the Primer3 program to amplify the ten loci in four multiplexes (Table S2, Supporting Information). One of these (AF528042.2) corresponds to the highly polymorphic CPFRAX6 locus described in Harbourne et al. (2005), but was monomorphic across all samples tested. Consequently, we screened a subset of our samples using the original CPFRAX6 primers, but these did not reveal any additional variation to that displayed using the AF528042.2 primers.

PCR was carried out in a total volume of 10 μl containing 100 ng genomic DNA, 5 pmol of 6-FAM- or HEX-labelled M13 primer, 0.5 pmol of M13-tailed forward primer, 5 pmol reverse primer, 1x PCR reaction buffer, 200 μM each dNTP, 2.5 mM $MgCl_2$ and 0.25 U GoTaq Flexi DNA polymerase (Promega, Sunnyvale, CA, USA). PCR was carried out on a MWG Primus thermal cycler (Ebersberg, Germany) using the following conditions: initial denaturation at 94 ^{o}C for 3 min followed by 40 cycles (30 for chloroplast loci) of denaturation at 94 ^{o}C for 30 s, annealing at 55 ^{o}C for 30 s, extension at 72 ^{o}C for 30 s, and a final extension at 72 ^{o}C for 5 min. Genotyping was carried out on an AB3730xl capillary genotyping system. (Applied Biosystems, Foster City, CA, USA). Allele sizes were scored using the GENEMAPPER software package (v4.1; Applied Biosystems) using LIZ-500 size standards, and were checked by comparison with previously sized control samples. Chromatograms were all inspected visually to check for large allele dropout (see Discussion).

158 *Data analysis –broad-scale*

159 GENEPOP (V3.4; Raymond and Rousset, 1995) was used to test for linkage disequilibrium
160 between nuclear microsatellite loci. To estimate genetic diversity within the populations,
161 levels of observed (H_O) and expected (H_E) heterozygosity, levels of allelic richness (A_R) and
162 fixation indices (F_{IS}) were calculated using the FSTAT software package (V2.9.3.2; Goudet,
163 2001). Significance of F_{IS} was determined by 10,000 randomisation steps. We also
164 estimated null allele frequencies using the CERVUS software package (V3.0.3; Kalinowski et
165 al. 2007), as previous studies using the same microsatellites (Femsatl-4, Femsatl-8, Femsatl-
166 11, Femsatl-16, Femsatl-19 and M230) have suggested the possibility of null alleles.
167 Chloroplast microsatellite allele sizes were combined into haplotypes, and levels of genetic
168 diversity (H) based on haplotype frequencies were calculated using the ARLEQUIN software
169 package (V3.5.1.2; Excoffier and Lischer, 2010).

170 The overall level of genetic differentiation between populations was estimated using Φ_{ST},
171 which gives an analogue of F_{ST} (Weir and Cockerham, 1984) calculated within the analysis
172 of molecular variance (AMOVA) framework (Excoffier et al. 1992) using ARLEQUIN. In
173 addition, as the high numbers of alleles and high levels of diversity associated with
174 microsatellite loci can lead to an underestimation of genetic differentiation between
175 populations, we also calculated Hedrick's G'_{ST} (Hedrick 2005) for the nuclear microsatellite
176 data set. To further identify possible patterns of genetic structure, the software package
177 BAPS (V5; Corander et al. [2003]) was used to identify clusters of genetically similar
178 populations using a Bayesian approach. Ten replicates were run for all possible values of the
179 maximum number of clusters (K) up to $K = 36$, the number of populations sampled, with a
180 burn-in period of 10,000 iterations followed by 100,000 iterations. Multiple independent
181 runs always gave the same outcome.

A test for isolation-by-distance **(**IBD; Rousset 1997) was carried out to test the null hypothesis of a stepping-stone model of gene flow between populations of *F. excelsior*. The ISOLDE test implemented in the GENEPOP software package was used to assess the relationship between genetic distance, measured as Hedrick's G'_{ST} (Hedrick 2005), and geographical distance between population pairs. 1,000 permutations were used for the Mantel test.

To test for spatial genetic structuring (SGS) within populations, which could give rise to Wahlund effects, we carried out spatial autocorrelation analyses using SPAGEDI (V1.4; Hardy and Vekemans, 2002). Mean coancestry coefficients (θ_{xy}; Loiselle et al. 1995) between pairs of individuals were calculated for both the 0-50 m and 50-100 m distance classes for each population, with the remaining size intervals (50 m to 500 m) reflecting the overall size of each population, and plotted as a correlogram, with 95% confidence intervals calculated from 1,000 permutations of individuals within each distance class, and for estimates of θ_{xy} using 1,000 permutations. Finally, for comparison of levels of SGS with other species, we calculated the *Sp* statistic of Vekemans and Hardy (2004) as $-b_k / (1 - \theta_1)$, where b_k is the slope of the regression of θ_{xy} against the logarithm of the distance, and θ_1 is the mean value of the pairwise coancestry coefficients calculated between all pairs of individuals within the first distance class (0-50 m).

Data analysis – fine-scale

Only seven nuclear microsatellite loci were used in the fine-scale study, since locus Femsat-19 exhibited alleles that differed by only a single base pair, and we wanted to ensure exact matches between putative parents and offspring. We employed two approaches to determine parentage of saplings. The first was a simple exclusion approach, based on the premise that we had sampled all potential adult parents in each stand. Any adult that did not have at least

one allele matching those exhibited by a sapling at all seven loci was excluded as a potential parent of that sapling. The second was a likelihood-based approach implemented in the CERVUS software package (V3.0; Kalinowski et al. 2007). This was used in addition to strict exclusion, since the program can allow for potential genotyping errors, and the fact that not all putative parents may have been sampled. Simulations were run for 10,000 iterations, with a genotyping error rate of 0.01, since we had manually scored all markers to check for automated miscalls and allelic dropout, and assuming 95% sampling of putative parents. Parent-pairs or individual parents were assigned based on the critical values for the 95% strict log-likelihood (LOD) scores.

Results

Broad-scale study

No evidence of linkage disequilibrium was detected between any of the eight nuclear microsatellites analysed. Between nine (FR646655) and 51 (M230) alleles were detected per locus, with a total of 261 (mean = 32.625 per locus; Table 2). Levels of observed (H_O) and expected (H_E) heterozygosity ranged from 0.442 (FR646655) to 0.909 (M230; mean = 0.709), and from 0.477 (FR646655) to 0.937 (M230; mean = 0.765), respectively. Levels of F_{IS} ranged from -0.004 (Femsatl-16) to 0.236 (Femsatl-8), with a mean value of 0.067. The estimated frequency of null alleles ranged from zero (Femsatl-16) to 0.142 (Femsatl-8), with a mean value of 0.041. The proportion of large alleles not called by the GENEMAPPER software under the default settings in the four loci where there was significant large allele dropout (Femsatl-4, Femsatl-8, Femsatl-11 and M230) ranged from 2.98% (Femsatl-4) to 11.59% (M230).

Within populations, levels of allelic richness (A_R) averaged over loci ranged from 9.52 (Glenarm Forest) to 11.52 (Killeter Forest), with a mean value of 10.53 (Table 1). A total of 41 private alleles was detected, with the number per population ranging from zero to four. The majority (38) of these were restricted to a single individual, with the remaining three being found in two individuals. Levels of observed (H_O) and expected (H_E) heterozygosity ranged from 0.637 (Castle Hill) to 0.823 (Glenarm Forest; mean = 0.709), and from 0.712 (Trassey Road) to 0.809 (Rostrevor and Randalstown; mean = 0.765) respectively. The heterozygote deficit observed in the majority of the populations gave rise to F_{IS} values which were significantly higher than zero in 27 of the 36 populations studied, ranging from 0.053 (Killeter Forest) to 0.168 (Letterfrack; mean = 0.067). Diversity statistics for individual loci by population are given in Table S3, Supporting Information, and indicate that significant F_{IS}

241 values were generally due to high values at locus Femsatl-8, which were significantly higher
242 than zero in 32 of the 36 population studied, suggesting the presence of null alleles at this
243 locus.

244 Five of the ten chloroplast microsatellite loci studied were polymorphic in the samples
245 analysed, exhibiting between two and four alleles (Table S1, Supporting Information).
246 Combining allele sizes across loci gave eight haplotypes (See Figure S1, Supporting
247 Information for a network of evolutionary relationships between haplotypes). One of these
248 (H1) was found in the vast majority (995 out of 1052) of the trees studied. Levels of
249 haplotype diversity (*H*) ranged from zero (several populations) to 0.572 (Barnett Demesne;
250 Table 1).

251 Levels of population differentiation were $\Phi_{ST} = 0.0131$ and Hedrick's $G'_{ST} = 0.0547$ for
252 the nuclear microsatellites, and $\Phi_{ST} = 0.2629$ for the chloroplast microsatellites (results of the
253 AMOVA are given in Table 3). The BAPS analysis assigned all 36 populations to a single
254 genetic cluster, although a weak but significant isolation-by-distance ($P = 0.005$) was
255 observed across all populations, but not across NI populations only (P = 0.09; Figure 3).
256 Finally, the spatial autocorrelation analyses revealed very little significant within-population
257 spatial genetic structuring, with structuring only observed up to 50 m in the Dromora,
258 Rostrevor, Randalstown and Lemnagore Wood populations (Figure S2, Supporting
259 Information), and *Sp* values ranging from 0.000 (several populations) to 0.020 (Knockninney
260 Hill; Table 1).

261

262 *Fine-scale study*

263 We successfully genotyped 140 adult trees and 93 saplings from Barnett Demesne, and 44
264 adults and 39 saplings from Cregagh Glen. For the Barnett Demesne stand, there was
265 extremely good agreement between parentage based on strict exclusion, and assignments

266 based on likelihood implemented in CERVUS: in only five cases, CERVUS identified a second
267 parent where strict exclusion only identified a single parent, and there were four cases where
268 a sapling/parent combination was identified by exclusion, but where the adult had a LOD
269 score below the threshold calculated by CERVUS. Based on the CERVUS results, a single
270 parent was identified for 42 saplings, both parents were identified for 41 saplings, and no
271 parent within the stand was identified for five saplings. Three putative parents above the
272 LOD threshold were identified for a single sapling, which was not included further in the
273 analysis, as were the four saplings where a parent was identified by exclusion, but this adult
274 had a LOD score below the threshold. Three chloroplast microsatellite haplotypes were
275 identified, and in the 41 cases where both parents were identified, differences in chloroplast
276 haplotypes between the parents allowed the identification of the seed parent in 13 cases.
277 Consequently, seed dispersal distances could be calculated for 55 of the saplings: 13 where
278 the seed parent was identified in the parent-pair, and for the 42 saplings where a single parent
279 was identified, since the assumption that a single parent is the seed parent is far more
280 parsimonious than the alternative explanation of the adult being the pollen parent, which
281 pollinated another tree outside the stand, with the seed subsequently dispersing back into the
282 stand. Furthermore, in all cases of single parent assignment, there was a match between the
283 adult and sapling chloroplast haplotype, consistent with the adult being the seed parent. This
284 includes the rarest haplotype, found in a single sapling and a single adult, which were classed
285 as parent-offspring pairs by both CERVUS and strict exclusion. Dispersal distances ranged
286 from 3 to 223 m (mean = 42 m; median = 31 m; Figure 4). Pollination distances were
287 calculated for the 13 parent-pairs, and ranged from 2 to 266 m (mean = 93 m; median = 83
288 m). Realized pollen dispersal distances i.e. from pollen parent to sapling ranged from 7 to
289 168 m (mean = 65 m; median = 47 m; Table 4).

290 In the Cregagh Glen stand, very few putative parents were identified by CERVUS which
291 had LOD scores above the critical value. This was due to a combination of lower overall
292 genetic variation, and the occurrence of high-frequency alleles at several of the microsatellite
293 loci. Four dispersal events from separate single parents were identified, with distances of 23,
294 82, 123 and 148 m. In eleven cases, however, no parent was identified, suggesting
295 immigration of seed into the stand. All adults and saplings shared a single chloroplast
296 microsatellite haplotype.

Discussion

Lack of genetic structure and implications for restorative planting

For over 50 years now, the concept of provenance has been integral to forestry practices, particularly with respect to restocking and / or replanting of woodlands (reviewed in Jones and Burley 1973). This reflects observed phenotypic and underlying genetic variation across species' distributions, and recommends that where possible, woodlands should be restocked with local seeds or seedlings. Our finding that ash woodlands across Ireland are genetically uniform suggests that the concept of provenance might more usefully reflect the geographic distribution of genetic variation, and that all could be treated as a single management unit, given the lack of genetic differentiation between populations and the low incidence of private alleles. The observed level of population differentiation based on nuclear microsatellites (Φ_{ST} = 0.0131) was the second lowest reported for European ash, with previous studies estimating between 1.2% (Hebel et al. 2006) and 8.7% (Heuertz et al. 2001) of nuclear diversity partitioned between populations, and is consistent with wind pollination and seed dispersal (Wardle 1961). Unfortunately, these previous studies did not calculate comparable statistics to the G'_{ST} value of 0.0547 observed in the present study, but future studies using microsatellites should calculate the equivalent statistic to take into account underestimation of levels of differentiation when using highly variable markers (Hedrick 2005).

Replacement of native trees for whatever reason should be based on knowledge of the geographic distribution of genetic variation (Godefroid et al. 2011). Our results clearly indicate that the source of material for replanting ash, at least in Ireland, is largely irrelevant, given such low levels of differentiation. The inclusion of samples from the extreme east (Co. Wicklow), south (Co. Cork) and west (Co. Galway) of Ireland in the same genetic cluster as the 33 populations from Northern Ireland indicates that our findings are probably applicable

322 to ash woodlands across the island of Ireland as a whole. Furthermore, the Irish samples of
323 ash exhibited similar levels of nuclear genetic diversity to those in Great Britain and
324 continental Europe, including populations from putative refugial areas that should harbour the
325 highest levels of variation (Heuertz et al. 2001; Morand et al. 2002; Heuertz et al. 2004a;
326 Heuertz et al. 2004b; Ferrazzini et al. 2007; Sutherland et al. 2010; Gérard et al. 2013).
327 However, such replanting should be carried out using native material from long-established,
328 unplanted / unstocked woodlands, since recent studies have shown that material used for
329 replanting in Ireland often contains individuals that possess alleles introgressed from the
330 congeneric *F. angustifolia* (Thomasset et al. 2013).
331 Levels of chloroplast genetic diversity were very low, with a single haplotype found in
332 almost 95% of all the individuals studied. This haplotype most likely corresponds to
333 Haplotype H04 from Heuertz et al. (2004a), which is also the dominant haplotype in Britain
334 as a result of postglacial recolonization from Iberia. Populations in the east of Northern
335 Ireland tended to exhibit the highest levels of chloroplast diversity, with most of the
336 populations in the west fixed for the most common haplotype. This could be due to founder
337 effects associated with westward recolonization from Britain or to predominantly eastward
338 seed dispersal by prevailing westerly winds, since the chloroplast genome is maternally
339 inherited in ash, or to a combination of both.
340 Several previous population genetic studies on ash have reported significant, and often
341 high, levels of F_{IS}, which have been attributed to various factors including inbreeding, null
342 alleles, large allele dropout and Wahlund effect (Morand et al. 2002; Ferrazzini et al. 2007;
343 Sutherland et al. 2010). The mean F_{IS} calculated for ash populations across Northern Ireland
344 (0.067) is significantly lower than that reported by Sutherland et al. (2010), who used six of
345 the eight loci analysed in the present study to examine populations throughout Great Britain
346 (mean $F_{IS} = 0.182$; Mann-Whitney test, $z = 6.07$, $P < 0.0001$). We designed new primers to

amplify previously characterized microsatellite loci with the aim of circumventing any potential problems with null alleles, and our estimated null allele frequencies were generally much lower than those reported in Sutherland et al. (2010). However, on genotyping a subset of our samples using the same primers as Sutherland et al. (2010), we did not observe an increase in F_{IS} or estimated null allele frequencies, suggesting that the previously observed homozygote deficiencies were not due to null alleles as a result of non-amplification. Furthermore, although Femsatl-8 exhibited the highest F_{IS} among the loci analysed in both cases, which might suggest null alleles associated with this particular marker, the same locus exhibited the lowest F_{IS} in a previous study in Germany (Hebel et al. 2006). The fact that this locus was significantly higher than zero in 32 of the 36 populations studied, even where the majority of the other alleles in these populations did not yield significant F_{IS} values, however, does suggest the presence of null alleles.

Another potential cause of heterozygote deficiency is large allele dropout, where short alleles are preferentially amplified during the PCR. Automated scoring software packages, such as GENOTYPER and GENEMAPPER, will often not identify these long alleles. We took the precaution of manually checking each chromatogram, and using the default settings for allele scoring in the GENEMAPPER package, we identified uncalled large alleles at frequencies ranging from 2.98% to 11.59% at four of the eight loci studied (see Figure S3, Supplementary information for an example). The phenomenon is particularly prevalent at loci with a high number of alleles over a large size range, and with such high frequencies of uncalled alleles, analyses based on the raw outputs from these genotyping packages would result in apparent homozygote excesses and subsequently inflated F_{IS} values.

Sutherland et al. (2010) suggested that the F_{IS} values observed in their study might be due to a Wahlund effect, namely the occurrence of spatial genetic structuring within populations, a scenario also proposed to a lesser degree by Ferrazzini et al. (2007). Because we recorded

372 GPS coordinates for each of the trees sampled in the present study, we were able to carry out
373 spatial autocorrelation analyses to test for such structuring. With the exception of
374 significantly higher levels of relatedness up to 50 m in only four populations out of the 36
375 studied, we found no evidence of Wahlund effects.

376 Given that we can exclude null alleles (with the possible exception of locus Femsatl-8,
377 which consistently exhibits high F_{IS} levels across most studies), large allele dropout and
378 Wahlund effects, the F_{IS} values observed would appear to give a true measure of the levels of
379 inbreeding in populations of ash in the present study. Our spatial autocorrelation analyses
380 found little evidence for the breeding "subunits" previously suggested to exist within ash
381 woodlands (Heuertz et al. 2001; Morand et al. 2002). The mean value of *Sp* calculated for
382 the 36 populations studied (0.006) was lower than the mean value reported for trees (0.010)
383 by Vekemans and Hardy (2004) and the mean value from six microsatellite-based studies in
384 tropical trees (0.017; Hardy et al. 2006), although higher than that calculated for a Romanian
385 population of *F. excelsior* (0.002; Heuertz et al. 2003). This may be due at least in part to the
386 differing densities of ash trees in the various woodlands analysed in the present study. For
387 example, the Knockninney Hill population, which presented the highest *Sp* value, had only a
388 single pair of individuals within 100 m of each other.

389

390 *Evidence for frequent long-distance dispersal events*

391 Although the use of molecular genetic techniques, particularly high-resolution microsatellite
392 markers, has provided valuable insights into seed dispersal in natural plant populations
393 (Powell et al. 1996; Ashley 2010), there still remain problems associated with identifying the
394 seed parents of established plants and / or seedlings in monoecious species. Estimates of
395 pollen-mediated gene flow can be relatively easily obtained by genotyping seeds and
396 "subtracting" the genotype of the maternal plant, thus leaving the paternal genotype which

397 can be matched to putative parent plants. Such an approach has been employed previously in
398 ash, indicating pollination events at distances over several hundred metres, and up to nearly 3
399 km (Bacles et al. 2005; Bacles and Ennos 2008; Thomasset et al. 2014). Estimating seed
400 dispersal, however, is a more difficult process, particularly in monoecious species (Sork and
401 Smouse 2006). Previous studies have attempted to genotype the endocarp tissue to identify
402 the maternal parent of dispersed seeds (Godoy and Jordano 2001; Garcia et al. 2007), but this
403 only provides estimates of initial dispersal, and does not necessarily provide an indication of
404 true population demography and recruitment (effective dispersal), for which identification of
405 the mothers of seedlings or established plants is necessary. For seedlings in a population of an
406 outcrossing species, it may be possible to identify both parents, one parent, which will be
407 either the pollen or seed parent, or neither parent, indicating immigration of seed into the
408 population. In angiosperms, chloroplast-specific markers can be used in conjunction with
409 biparentally inherited nuclear markers to assign the maternal and the paternal parent where
410 both parents are identified, since the chloroplast genome is usually maternally inherited. One
411 drawback of such an approach is the low mutation rate in chloroplast genomes, meaning that
412 often there is inadequate resolution to assign the maternal parent (Wolfe et al. 1987). By
413 using highly polymorphic chloroplast microsatellite markers, which allow the high-resolution
414 of maternal genotypes, it may often be possible to determine which is the mother plant in
415 cases where both parents are identified using nuclear microsatellites (Provan et al. 2001).
416 By using a combination of nuclear and chloroplast microsatellite markers, we were able to
417 assign seed and pollen parents unambiguously for 13 out of 41 saplings for which both
418 parents were identified within the Barnett Demesne stand, as well as assigning putative seed
419 parents to a further 42 saplings. Previous studies using genetic markers to identify the source
420 of established seedlings relied on genotyping any maternal tissue associated with the
421 seedling, but these approaches can be problematic due to the low quality of DNA typically

422 recovered from the pericarp (Grivet et al. 2009; Smouse et al. 2012). Chloroplast
423 microsatellite markers provide a convenient, high-resolution, uniparental assay (maternal in
424 the majority of angiosperms and paternal in the majority of gymnosperms) that can be run on
425 leaf material from established plants, and thus allow the assignment of seed and pollen
426 parents where both parents are identified (Provan et al. 2001; Ebert and Peakall 2009a),
427 circumventing the need to rely on genotypes from maternal tissues. Primers to amplify
428 chloroplast microsatellites are available for a wide range of species, and the high levels of
429 conservation of the chloroplast genome means that primers developed for a particular species
430 often give polymorphic markers in related taxa (Provan et al. 2001). In addition, sets of
431 universal primers are available to facilitate *de novo* development of these markers,
432 particularly for non-model organisms or taxa with little sequence information available in
433 DNA sequence databases such as GenBank (e.g. Ebert and Peakall 2009b).
434 Our findings indicate frequent seed dispersal over distances greater than 100 m, with six
435 known within-stand dispersal events (over 10 %) exceeding this range. We also identified
436 immigration into the stand in 16 cases (five out of 93 [5%] from Barnett Demesne and eleven
437 out of 39 [28%] from Cregagh Glen). Barnett Demesne is located in a largely urban area, and
438 the nearest stand of ash trees was 400 m to the east, at Shaw's bridge (Fig. 1), suggesting that
439 this was the minimum dispersal distance of immigrant seed into the stand. Despite the lower
440 genetic diversity in the Cregagh Glen population, which led to a lower level of parentage
441 assignment, the higher rate of immigration appeared to result from extra-stand fertilization.
442 Cregagh Glen is in a more agriculture-dominated landscape on the eastern edge of Belfast,
443 and it is possible that immigrant seed dispersed from neighbouring isolated individuals, low-
444 density hedgerow trees, or from the next-nearest substantial stand of ash, which was a
445 similarly-sized stand in a ravine *ca.* 500 m to the east (Fig. 1).

446 Only two previous genetic studies on seed dispersal in ash have been carried out. Heuertz
447 et al. (2003) used simulation studies coupled with estimates of kinship from five biparentally
448 inherited nuclear microsatellites, including four of the seven loci used in the present study, to
449 infer levels of seed and pollen dispersal in a continuous forest in southeast Romania. The
450 estimated levels of seed dispersal, which were ≤ 14 m, were lower than both the mean and
451 median values calculated for the Barnett Demesne stand in the present study, and far lower
452 than the majority of individual events identified. Bacles et al. (2006) used a direct,
453 individual-based approach to assess seed dispersal in a highly fragmented landscape in
454 southern Scotland. They detected multiple long-distance events, often between fragments of
455 up to 1.4 km, but this is most likely due to the chronically fragmented nature of their study
456 site, with far fewer barriers to dispersal, and the increased chance of the winged seeds being
457 uplifted in the initial stages of dispersal. This scenario is very different to the closed, semi-
458 continuous woodlands analysed in the present study, and our findings may better reflect
459 patterns of dispersal in more typical mixed-deciduous woodlands. Interestingly, since Bacles
460 et al. (2006) had no means to identify the seed parent where parent pairs were identified, they
461 assumed that the closer of the two to the seedling was the seed parent, but our analysis
462 indicated that the seed parent was the more distant parent of the two in four out of twelve
463 cases (and in one case they were equidistant). This further highlights the utility and
464 importance of our approach in unambiguously identifying maternal and paternal parents to
465 accurately quantify dispersal.
466 The ability to identify the seed sources of established plants allows insights into the end-
467 results of dispersal in population demography i.e. initial dispersal followed by germination
468 and survival / recruitment into the population. This means that post-dispersal processes, such
469 as competition and density-dependent mortality, can be addressed. This was not possible in
470 early genetic studies on dispersal, which relied on genotyping seeds, and thus could only

assess initial seed dispersal (e.g. Godoy and Jordano 2001; Ziegenhagen et al. 2003; Grivet et al. 2005). Although we did not specifically test for such effects, our plot of effective seed dispersal distances within Barnett Demesne is consistent with a Janzen-Connell recruitment process (Janzen 1970; Connell 1971; Augspurger 1983). Dispersal in the stand peaked at 30 – 40 m, before tailing off quickly, suggesting density-dependent mortality close to the mother plant. A similar pattern was observed in a genetic study on Aleppo pine (*Pinus halepensis*) specifically designed to test for Janzen-Connell effects (Steinitz et al. 2011).

Conclusions

Our results suggest that although there is considerable genetic variation in ash trees across the whole of Ireland, there is no evidence of population genetic structure. Hence, the imposition of "seed zones" as part of a recovery plan for ash trees in the aftermath of near total mortality due to ash dieback may not be justified, and is an avoidable cost. Our findings of frequent, long-distance dispersal events have further implications for the survival and persistence of ash woodlands in the face of a range of threats. Infection by the causal agent of ash dieback, *Chalara fraxinea*, may lead to loss of woodlands, either by pathogenic mortality or by anthropogenic clearance as a means of control (Pautasso et al. 2013). The high capacity for dispersal indicated by our results suggests good potential for natural regeneration, as well as for the spread of resistance to the disease, both via seeds and via pollen-mediated gene flow from individuals exhibiting inherent resistance. In addition, high levels of migration will be necessary to respond to global climate change, although this is very much dependent on the rate and extent of these changes.

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493

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Data archiving statement

All data will be deposited in DRYAD on acceptance.

504 **References**

505

506 Ashley MV (2010) Plant parentage, pollination and dispersal: how DNA microsatellites have
507 altered the landscape. Critical Rev Plant Sci 29:148-161.
508 Augspurger C (1983) Recruitment around tropical trees: changes in cohort distance with
509 time. Oikos 40:189-196.
510 Bacles CFE, Burczyk J, Lowe AJ, Ennos RA (2005) Historical and contemporary mating
511 patterns in remnant populations of the forest tree *Fraxinus excelsior* L. Evolution 59:979-
512 990.
513 Bacles CFE, Lowe AJ, Ennos RA (2006) Effective seed dispersal across a fragmented
514 landscape. Science 311:628.
515 Bacles CFE, Ennos RA (2008) Paternity analysis of pollen-mediated gene flow for *Fraxinus*
516 *excelsior* L. in a chronically fragmented landscape. Heredity 101:368-380.
517 Brachet S, Jubier MF, Richard M, Jung-Muller B, Frascaria-Lacoste N (1999) Rapid
518 identification of microsatellite loci using 5' anchored PCR in common ash *Fraxinus*
519 *excelsior*. Mol Ecol Notes 8:160-163.
520 Connell JH (1971) On the role of natural enemies in preventing competitive exclusion in
521 some marine animals and forest trees. In den Boer PJ, Gradwell GR (eds) Dynamics of
522 Populations. Centre for Agricultural Publishing and Documentation. Wageningen, The
523 Netherlands pp. 298-312.
524 Corander J, Waldmann P, Sillanpää MJ (2003) Bayesian analysis of genetic differentiation
525 between populations. Genetics 163:367-374.
526 DeSalle R. Amato G (2004) The expansion of conservation genetics. Nature Rev Genet
527 5:702-712.

528 Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf
529 tissue. Phytochem Bull 19: 1-15.
530 Ebert D, Peakall R (2009a) Chloroplast simple sequence repeats (cpSSRs): technical
531 resources and recommendations for expanding cpSSR discovery and applications to a
532 wide array of plant species. Mol Ecol Resources 9:673-690.
533 Ebert D, Peakall R (2009b) A new set of universal *de novo* sequencing primers for extensive
534 coverage of noncoding chloroplast DNA: new opportunities for phylogenetic studies and
535 cpSSR discovery. Mol Ecol Resources 9:777-783.
536 Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from
537 metric distances among DNA haplotypes - application to human mitochondrial DNA
538 restriction data. Genetics 131:479-491.
539 Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform
540 population genetics analyses under Linux and Windows. Molecular Ecol Resources
541 10:564-567.
542 Ferrazzini D, Monteleoni I, Belletti P (2007) Genetic variability and divergence among
543 Italian populations of common ash (*Fraxinus excelsior* L.). Annals Forest Sci 64:159-168.
544 Forget PM, Wenny D (2005) How to elucidate seed fate? A review of methods used to study
545 seed removal and secondary seed dispersal. In: Forget PM et al. (eds) Seed Fate: Seed
546 Predation, Seed Dispersal and Seedling Establishment. CABI Publishing, Wallingford,
547 UK pp. 379-393.
548 FRAXIGEN (2005) *Ash species in Europe: Biological Characteristics and Practical*
549 *Guidelines for Sustainable Use*. Oxford Forestry Institute, Oxford, UK.
550 Garcia C, Jordano P, Godoy JA (2007) Contemporary pollen and seed dispersal in a *Prunus*
551 *mahaleb* population: patterns in distance and direction. Mol Ecol 16:1947-1955.

552 Godoy JA, Jordano P (2001) Seed dispersal by animals: exact identification of source trees
553 with endocarp DNA microsatellites. Mol Ecol 10:2275-2283.
554 Gérard PR, Temunovic M, Sannier J, Bertolino P, Dufour J, Frascaria-Lacoste N, Fernández-
555 Manjarrés JF (2013) Chilled but not frosty: understanding the role of climate in the
556 hybridization between the Mediterranean *Fraxinus angustifolia* Vahl and the temperate
557 *Fraxinus excelsior* L. (Oleaceae) ash trees. J Biogeogr 40:835-846.
558 Godefroid S, Piazza C, Rossi G. *et al.* (2011) How successful are plant species
559 reintroductions? Biol Conserv 144:672-682.
560 Goudet J (2001) FSTAT, version 2.9.3, A program to estimate and test gene diversities and
561 fixation indices. http://www2.unil.ch/popgen/softwares/fstat.htm.
562 Grivet D, Robledo-Arnuncio JJ, Smouse PE, Sork VL (2009) Relative contribution of
563 contemporary pollen and seed dispersal to the neighbourhood size of a seedling population
564 of California valley oak (*Quercus lobata*, Née). Mol Ecol 16:3967-3979.
565 Harbourne ME, Douglas GC, Waldren S, Hodkinson TR (2005) Characterization and primer
566 development and amplification of chloroplast microsatellite regions of *Fraxinus excelsior.*
567 J Plant Res 118:339-341.
568 Hardy OJ, Vekemans X (2002) SPAGeDi: a versatile computer program to analyse spatial
569 genetic structure at the individual or population levels. Mol Ecol Notes 2:618-620.
570 Hardy OJ, Maggia L, Bandou E, Breyne P, Caron J, Chevallier MH, Doligez A, Dutech C,
571 Kremer A, Latouche-Hallé C, Troispoux V, Veron V, Degen B (2006) Fine-scale genetic
572 structure and gene dispersal inferences in 10 neotropical tree species. Mol Ecol 15:559-
573 571.
574 Hebel I, Haas R, Dounavi A (2006) Genetic variation of common ash (*Fraxinus excelsior* L.)
575 populations from provenance regions in southern Germany by using nuclear and
576 chloroplast microsatellites. Silvae Genetica 55:38-44.

577 Hedrick PW (2005) A standardized genetic differentiation measure. Evolution 59:1633-
578 1638.
579 Herbert R, Samuel S, Pattison G (1999) *Using Local Stock for Planting Native Trees and*
580 *Shrubs*. Forestry Commission Practice Note 8. Forestry Commission, Edinburgh, UK.
581 Heuertz M, Hausman J-F, Tsvetkov I, Frascaria-Lacoste N, Vekemans X (2001) Assessment
582 of genetic structure within and among Bulgarian populations of common as (*Fraxinus*
583 *excelsior* L.). Mol Ecol 10:1615-1623.
584 Heuertz M, Vekemans X, Hausman J-F, Palada M, Hardy OJ (2003) Estimating seed vs.
585 Pollen dispersal from spatial genetic structure in the common ash. Mol Ecol 12:2483-
586 2495.
587 Heuertz M, Fineschi S, Anzidei M *et al.* (2004a) Chloroplast DNA variation and postglacial
588 recolonization of common ash (*Fraxinus excelsior* L.) in Europe. Mol Ecol 13:3437-3452.
589 Heuertz M, Hausman J-F, Hardy OJ, Vendramin GG, Frascaria-Lacoste N, Vekemans X
590 (2004b) Nuclear microsatellites reveal contrasting patterns of genetic structure between
591 western and southeastern European populations of the common ash (*Fraxinus excelsior*_.
592 Evolution 58:976-988.
593 Howe HF, Smallwood J (1982) Ecology of seed dispersal. Annu Rev Ecol Syst 13:201-228.
594 Janzen D (1970) Herbivores and the number of tree species in tropical forests. Am Nat
595 104:501-528.
596 Jones N, Burley J (1973) Seed certification, provenance nomenclature and genetic history in
597 forestry. Silvae Genetica 23:53-58.
598 Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS
599 accommodates genotyping error increases success in paternity assignment. Mol Ecol
600 16:1099-1106.

601 Kowalski T (2006) *Chalara fraxinea* sp nov associated with dieback of ash (*Fraxinus*
602 *excelsior*) in Poland. Forest Pathol 36:264-270.
603 Lefort F, Brachet S, Frascaria-Lacoste N, Edwards KJ, Douglas GC (1999) Identification and
604 characterization of microsatellite loci in ash (*Fraxinus excelsior* L.) and their conservation
605 in the olive family. Mol Ecol Notes 8:1088-1090.
606 Levine JM, Murrell DJ (200) The community-level consequences of seed dispersal patterns.
607 Annu Rev Ecol Evol Syst 34:549-574.
608 Loiselle BA, Sork VL, Nason J, Graham C (1995) Spatial genetic structure of a tropical
609 understorey shrub, *Psychotria officinalis* (Rubiaceae). Am J Bot 82:1420-1425.
610 Marigo G, Peltier J-P, Girel J, Pautou G (2001) Success in the demographic expansion of
611 *Fraxinus excelsior* L. Trees 15:1-13.
612 Morand ME, Brachet S, Rossignol P, Dufour J, Frascaria-Lacoste N (2002) A generalised
613 heterozygote deficiency assessed with microsatellites in French common ash populations.
614 Mol Ecol 11:377-385.
615 Nathan R, Muller-Landau HC (2000) Spatial patterns of seed dispersal, their determinants
616 and consequences for recruitment. Trends Ecol Evol 15:278-285.
617 Pautasso M, Aas G, Queloz V, Holdenreider O (2013) European as (*Fraxinus excelsior*)
618 dieback – a conservation biology challenge. Biol Conserv 158:37-49.
619 Powell W, Machray GC, Provan J (1996) Polymorphism revealed by simple sequence
620 repeats. Trends Plant Sci 1:215-222.
621 Provan J, Powell W, Hollingsworth PM (2001) Chloroplast microsatellites: new tools for
622 studies in plant ecology and systematic. Trends Ecol Evol 16:142-147.
623 Queloz V, Grüning CR, Berndt R, Kowalski T, Sieber TN, Holdenreider O (2011) Cryptic
624 speciation in *Hymenoscyphus albidus*. Forest Pathol 41:133-142.

625 Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetic software for exact
626 tests and ecumenicism. J Hered 86:248-249.
627 Rousset F (1997) Genetic differentiation and estimation of gene flow from *F*-statistics under
628 isolation by distance. Genetics 145:1219-1228.
629 Smouse PE, Sork VL, Scofield DG, Grivet D (2012) Using seedling and pericarp tissues to
630 determine maternal parentage of dispersed valley oak recruits. J Hered 103:250-259.
631 Sork VL Smouse PE (2006) Genetic analysis of landscape connectivity in tree populations.
632 Landscape Ecol 21:821-836.
633 Steinitz O, Troupin D, Vendramin GG, Nathan R (2011) Genetic evidence for a Janzen-
634 Connell recruitment pattern in reproductive offspring of *Pin us halepensis* trees. Mol Ecol
635 20:4152-4164.
636 Sutherland BG, Belaj A, Nier S, Cottrell JE, Vaughan SP, Hubert J, Russell K (2010)
637 Molecular biodiversity and population structure in common ash (*Fraxinus excelsior* L.) in
638 Britain: implications for conservation. Mol Ecol 19:2196-2211.
639 Thomasset M, Fernández-Manjarrés JF, Douglas GC, Bertolino P, Frascaria-Lacoste N,
640 Hodkinson TR (2013) Assignment testing reveals multiple introduced source populations
641 including potential ash hybrids (*Fraxinus excelsior* x *F. angustifolia*) in Ireland. Eur J
642 Forest Res 132:195-209.
643 Thomasset M, Hodkinson TR, Restoux G, Frascaria-Lacoste N, Douglas GC, Fernández-
644 Manjarrés JF (2014) Thank you for not flowering: conservation genetics and gene flow
645 analysis of native and non-native populations of *Fraxinus* (Oleaceae) in Ireland. Heredity
646 112:596-606.
647 Vekemans X, Hardy OJ (2004) New insights from fine-scale spatial genetic structure analysis
648 in plant populations. Mol Ecol 13:921-935.
649 Wang BC, Smith TB (2002) Closing the seed dispersal loop. Trends Ecol Evol 17:379-385.

650 Wardle P. 1961. Biological flora of the British Isles: *Fraxinus excelsior* L. J Ecol 49:739-
651 751.
652 Webber J (1981) A natural biological control of Dutch elm disease. Nature 292:449-451.
653 Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population
654 structure. Evolution 38:1358-1370.
655 Wolfe KH, Li W-H, Sharp PM (1987) Rates of nucleotide substitution vary greatly among
656 plant mitochondrial, chloroplast and nuclear DNAs. Proceedings Natl Acad Sci USA
657 84:9054-9058.
658 Ziegenhagen B, Liepelt S, Kuhlenkamp V, Fladung M (2003) Molecular identification of
659 individual oak and fir trees from maternal tissues of their fruits or seeds. Trees 17:345-
660 350.

Table 1 Details of populations studied. N – number of individuals analysed; A_R – allelic richness; P – number of private alleles; H_O – observed heterozygosity; H_E – expected heterozygosity; F_{IS} – inbreeding coefficient; H1-H8 – frequency of chloroplast haplotypes; H – gene diversity.

No	Name	Lat	Long	Nuclear							Chloroplast									
		(N)	(W)	N	A_R	P	H_O	H_E	F_{IS}	Sp^a	N	H1	H2	H3	H4	H5	H6	H7	H8	H
1	Portaferry	54.391	5.565	30	9.80	-	0.673	0.750	0.104^{***}	0.000	30	28	2	-	-	-	-	-	-	0.129
2	Downpatrick	54.352	5.700	30	10.16	-	0.647	0.734	0.121^{***}	0.003	30	30	-	-	-	-	-	-	-	0.000
3	Helen's Bay	54.672	5.731	30	10.17	2	0.656	0.730	0.103^{***}	0.005	29	26	3	-	-	-	-	-	-	0.192
4	Magheramourne	54.810	5.781	30	10.24	-	0.782	0.784	0.003^{NS}	0.001	30	30	-	-	-	-	-	-	-	0.000
5	Glenarm Forest	54.962	5.958	30	9.52	2	0.823	0.770	-0.070^{NS}	0.008	30	29	-	1	-	-	-	-	-	0.067
6	Barnett Demesne	54.552	5.960	29	10.11	-	0.733	0.759	0.034^{NS}	0.001	30	12	16	-	-	1	1	-	-	0.572
7	Trassey Road	54.219	5.984	29	10.14	-	0.662	0.712	0.072^{**}	0.010	26	26	-	-	-	-	-	-	-	0.000
8	Dromara	54.330	5.996	29	11.42	1	0.723	0.782	0.078^{**}	0.011	29	29	-	-	-	-	-	-	-	0.000
9	Hillsborough	54.459	6.083	30	10.80	-	0.680	0.780	0.130^{***}	0.007	28	19	9	-	-	-	-	-	-	0.452
10	Glenariff Forest	55.016	6.100	30	10.85	-	0.727	0.775	0.063^{*}	0.010	29	28	-	-	-	-	-	1	-	0.069
11	Rostrevor	54.095	6.191	30	10.54	1	0.731	0.809	0.016^{NS}	0.013	26	26	-	-	-	-	-	-	-	0.000
12	Ballycastle Forest	55.174	6.226	30	9.82	1	0.755	0.757	0.003^{NS}	0.016	28	28	-	-	-	-	-	-	-	0.000
13	Randalstown	54.733	6.320	30	11.39	2	0.731	0.809	0.097^{***}	0.015	28	28	-	-	-	-	-	-	-	0.000
14	Portglenone	54.863	6.472	30	10.41	3	0.739	0.802	0.080^{**}	0.000	30	28	1	-	-	-	-	-	1	0.131
15	Gosford Park	54.303	6.522	30	11.07	2	0.738	0.771	0.044^{NS}	0.007	30	29	1	-	-	-	-	-	-	0.067
16	Ballymoney	55.062	6.560	30	9.90	1	0.697	0.771	0.098^{***}	0.000	28	28	-	-	-	-	-	-	-	0.000
17	Peatlands Park	54.486	6.616	29	10.42	3	0.667	0.730	0.086^{**}	0.012	29	26	-	-	3	-	-	-	-	0.192
18	Carndaisy Woods	54.749	6.725	30	10.31	1	0.690	0.774	0.110^{***}	0.000	30	30	-	-	-	-	-	-	-	0.000
19	Downhill	55.160	6.807	29	10.33	1	0.656	0.765	0.145^{***}	0.007	27	27	-	-	-	-	-	-	-	0.000
20	Drum Manor	54.639	6.815	30	11.34	3	0.727	0.779	0.068^{**}	0.003	30	29	1	-	-	-	-	-	-	0.067

Table 1 (Continued)

No	Name	Lat (N)	Long (W)	Nuclear							Chloroplast									
				N	A_R	*P*	H_O	H_E	F_{IS}	*Sp*[a]	*N*	H1	H2	H3	H4	H5	H6	H7	H8	*H*
21	Lemnagore Wood	54.331	6.841	29	10.18	-	0.714	0.720	0.008^{NS}	0.012	30	30	-	-	-	-	-	-	-	0.000
22	Roe Valley	55.025	6.938	30	9.93	-	0.669	0.777	0.141^{***}	0.015	30	29	-	1	-	-	-	-	-	0.067
23	Knockmany Forest	54.436	7.170	30	10.72	1	0.800	0.782	-0.023^{NS}	0.003	30	30	-	-	-	-	-	-	-	0.000
24	Slieve Beagh	54.380	7.203	28	10.92	-	0.670	0.733	0.088^{**}	0.006	30	28	-	2	-	-	-	-	-	0.129
25	Stranbane Glen	54.836	7.443	30	10.99	4	0.708	0.773	0.084^{***}	0.002	30	30	-	-	-	-	-	-	-	0.000
26	Crom	54.170	7.451	30	10.32	1	0.669	0.755	0.115^{***}	0.009	30	30	-	-	-	-	-	-	-	0.000
27	Knockninny Hill	54.231	7.573	28	11.15	3	0.691	0.770	0.103^{***}	0.020	30	30	-	-	-	-	-	-	-	0.000
28	Sloughan Glen	54.615	7.574	29	9.82	-	0.697	0.745	0.065^{*}	0.004	30	30	-	-	-	-	-	-	-	0.000
29	Castle Hill	54.484	7.722	30	11.14	1	0.637	0.757	0.161^{***}	0.000	30	30	-	-	-	-	-	-	-	0.000
30	Ely Lodge	54.412	7.725	30	11.39	3	0.728	0.776	0.062^{**}	0.011	30	30	-	-	-	-	-	-	-	0.000
31	Killeter Forest	54.687	7.744	30	11.52	1	0.738	0.779	0.053^{*}	0.001	30	30	-	-	-	-	-	-	-	0.000
32	Marble Arch	54.267	7.810	28	10.24	1	0.710	0.763	0.071^{**}	0.008	30	30	-	-	-	-	-	-	-	0.000
33	Castle Caldwell	54.493	7.965	30	11.21	2	0.687	0.742	0.075^{**}	0.000	30	30	-	-	-	-	-	-	-	0.000
34	Glenasmole Valley	53.251	6.371	28	11.00	1	0.762	0.776	0.018^{NS}	0.007	30	19	11	-	-	-	-	-	-	0.481
35	Knocknamallavoge	51.853	8.527	29	9.63	-	0.746	0.802	0.071^{*}	0.000	29	29	-	-	-	-	-	-	-	0.000
36	Letterfrack	53.553	9.948	26	10.02	-	0.644	0.771	0.168^{***}	0.005	26	24	2	-	-	-	-	-	-	0.148

[a] *Sp* is a measure of spatial genetic structure proposed by Vekemans and Hardy (2004). See Materials and Methods for details.

Table 2 Nuclear microsatellite loci analyzed in this study. N – number of alleles; H_O – observed heterozygosity; H_E – expected heterozygosity; F_{IS} – inbreeding coefficient; Null – null allele frequency; LAD – large allele dropout.

Locus	Primers*	N	Range (bp)	H_O	H_E	F_{IS}	Null	%LAD
FR639485	TGACAAACCCCAGCCTAACTCT GCCTGAGCAAGTAAAGACGCTA	21	310-348	0.613	0.629	0.024	0.019	-
FR646655	TGGAGCAGTTGAAGCACTGAAA TCTTCATCTTCCCAACAGCAGC	9	200-230	0.442	0.477	0.075	0.040	-
Femsatl-4	TTCATGCTTCTCCGTGTCTCAG GGGTGAAGAGGCTTTGTGTCAT	48	134-220	0.851	0.877	0.030	0.017	2.98
Femsatl-8	TTGCCTTTGTAGCTCAGG GCGTTGTCCTTAACTTTTCA	32	165-229	0.695	0.910	0.236	0.142	3.38
Femsatl-11	TGAACACAGCTCTTGACTCTGA GTTCTACTACTTCAAGAACAGGGGG	38	188-264	0.858	0.884	0.029	0.019	6.95
Femsatl-16	TGATCTCGTCCGAATTCACTGC ATGATGGCGACTTTTGGTGTGA	13	193-225	0.500	0.499	-0.004	N/A	-
Femsatl-19†	TCAAATTCCTGATTTCAGGGGGA CGCGTATGATGGTCTTTATCTCTGT	49	137-217	0.801	0.905	0.116	0.068	-
M230	ACGCGCACGTTCTTTCTATTTG GCTTTCTTGACCGGCTGACTAT	51	214-328	0.909	0.937	0.030	0.019	11.59

* Forward tailed with CACGACGTTGTAAAACGAC; Reverse tailed with GTGTCTT

† Not used in fine-scale study

Table 3 Analysis of molecular variance (AMOVA).

Markers	Source of variation	Sum of squares	Variance	% variation
Nuclear	Among populations	183.471	0.03917	1.31
	Within populations	6114.600	2.93971	98.69
Chloroplast	Among populations	15.373	0.01370	26.29
	Within populations	39.066	0.03841	73.71

Table 4 Pollination distances and realized pollen dispersal distances i.e. from father to sapling for the 13 saplings where the maternal parent was identified in the parent-pair**.**

Sapling	Father	Mother	Distance (m)	
			Pollination	Realized
BS-03	BA-006	BA-056	83	67
BS-08	BA-001	BA-009	2	8
BS-10	BA-005	BA-015	22	9
BS-18	BA-083	BA-054	158	149
BS-34	BA-105	BA-026	190	168
BS-50	BA-033	BA-017	12	7
BS-52	BA-032	BA-081	167	23
BS-55	BA-076	BA-025	68	100
BS-62	BA-135	BA-068	108	135
BS-65	BA-037	BA-006	22	41
BS-70	BA-076	BA-092	266	47
BS-72	BA-109	BA-116	2	75
BS-83	BA-135	BA-074	112	13

Table S1 Coordinates for samples analysed in the present study

Location	Sample	Lat	Long
Barnett Demesne	BA-001	54.55184233	-5.961468454
(Adults)	BA-002	54.55175997	-5.961379820
	BA-003	54.55189750	-5.961542988
	BA-004	54.55175360	-5.961534711
	BA-005	54.55183309	-5.961453458
	BA-006	54.55174566	-5.961596936
	BA-007	54.55175151	-5.961411156
	BA-008	54.55181722	-5.961577908
	BA-009	54.55183309	-5.961453458
	BA-010	54.55162413	-5.960783766
	BA-011	54.55179692	-5.961439805
	BA-012	54.55170506	-5.961320730
	BA-013	54.55171378	-5.961304838
	BA-014	54.55163402	-5.961370647
	BA-015	54.55165146	-5.961338862
	BA-016	54.55167813	-5.961322074
	BA-018	54.55176764	-5.961302150
	BA-019	54.55178326	-5.961162256
	BA-020	54.55186562	-5.961250890
	BA-021	54.55166655	-5.961168080
	BA-022	54.55192624	-5.960583199
	BA-023	54.55163064	-5.961169871
	BA-024	54.55162088	-5.961123987
	BA-025	54.55163220	-5.961262537
	BA-026	54.55156988	-5.961296561
	BA-027	54.55164222	-5.961323866
	BA-028	54.55161399	-5.961247989
	BA-029	54.55161477	-5.961294321
	BA-030	54.55156936	-5.961265673
	BA-031	54.55157066	-5.961342894
	BA-032	54.55164300	-5.961370199
	BA-033	54.55164326	-5.961385643
	BA-034	54.55161581	-5.961356098
	BA-035	54.55158094	-5.961419667
	BA-036	54.55178091	-5.961023257
	BA-037	54.55189658	-5.960955656
	BA-038	54.55170206	-5.960609846
	BA-039	54.55179743	-5.960404141
	BA-040	54.55149572	-5.961161147
	BA-041	54.55205048	-5.959958702
	BA-042	54.55192233	-5.960351534
	BA-043	54.55188486	-5.960260661
	BA-044	54.55184921	-5.960277898
	BA-045	54.55195537	-5.960179854
	BA-046	54.55185923	-5.960339227
	BA-047	54.55188642	-5.960353327
	BA-048	54.55178935	-5.959925369
	BA-049	54.55206348	-5.959664362
	BA-050	54.55209380	-5.959863795

Table S1 (continued)

Location	Sample	Lat	Long
Barnett Demesne (Adults)	BA-051	54.551708	-5.961574
	BA-052	54.55170531	-5.960269625
	BA-053	54.55199792	-5.960038614
	BA-054	54.55188642	-5.960353327
	BA-055	54.55194405	-5.960041303
	BA-056	54.55192233	-5.960351534
	BA-057	54.55197840	-5.959946844
	BA-058	54.55188642	-5.960353327
	BA-059	54.55189436	-5.960291101
	BA-060	54.55186820	-5.960338779
	BA-061	54.55170479	-5.960238736
	BA-062	54.55194535	-5.960118525
	BA-063	54.55178037	-5.959925817
	BA-064	54.55197203	-5.960101736
	BA-065	54.55165	-5.960217
	BA-066	54.550867	-5.960833
	BA-067	54.55165	-5.960233
	BA-068	54.5515	-5.960617
	BA-069	54.5516	-5.96065
	BA-070	54.551883	-5.961233
	BA-071	54.550917	-5.960883
	BA-072	54.551	-5.96145
	BA-073	54.551217	-5.96175
	BA-074	54.550633	-5.9616
	BA-075	54.5516	-5.9608
	BA-076	54.551033	-5.9615
	BA-077	54.5519	-5.96125
	BA-078	54.551783	-5.95995
	BA-079	54.5518	-5.959883
	BA-080	54.551983	-5.958883
	BA-081	54.55205	-5.958883
	BA-082	54.55425	-5.960983
	BA-083	54.5531	-5.96165
	BA-084	54.55268	-5.9602
	BA-085	54.55295	-5.961483
	BA-086	54.5541	-5.961
	BA-087	54.55417	-5.9612
	BA-088	54.554	-5.961867
	BA-089	54.55312	-5.9617
	BA-090	54.55293	-5.9613
	BA-091	54.55465	-5.957533
	BA-092	54.55212	-5.957833
	BA-093	54.55328	-5.957567
	BA-094	54.55662	-5.956933
	BA-095	54.55615	-5.95775
	BA-096	54.55648	-5.95715
	BA-097	54.55482	-5.959767
	BA-098	54.5531	-5.961717
	BA-099	54.55355	-5.962033
	BA-100	54.55255	-5.958633

Table S1 (continued)

Location	Sample	Lat	Long
Barnett Demesne (Adults)	BA-101	54.55208	-5.9579
	BA-102	54.5532	-5.961767
	BA-103	54.5525	-5.957933
	BA-104	54.55667	-5.956883
	BA-105	54.55325	-5.96185
	BA-106	54.55333	-5.961967
	BA-107	54.55247	-5.959067
	BA-108	54.55075	-5.9604
	BA-109	54.55063	-5.960217
	BA-110	54.55063	-5.960367
	BA-111	54.55133	-5.961183
	BA-112	54.55077	-5.960083
	BA-113	54.55067	-5.960067
	BA-116	54.55063	-5.960183
	BA-117	54.55068	-5.959883
	BA-118	54.55068	-5.960067
	BA-120	54.55063	-5.959883
	BA-121	54.5507	-5.959917
	BA-122	54.55055	-5.959883
	BA-124	54.55068	-5.959633
	BA-125	54.55063	-5.959567
	BA-126	54.55055	-5.959483
	BA-128	54.55062	-5.959567
	BA-129	54.55057	-5.959483
	BA-130	54.55057	-5.959417
	BA-131	54.55055	-5.959467
	BA-132	54.55055	-5.959433
	BA-133	54.55068	-5.959733
	BA-134	54.55065	-5.960217
	BA-135	54.55063	-5.959867
	BA-136	54.5508	-5.960317
	BA-137	54.55085	-5.961217
	BA-139	54.55075	-5.96015
	BA-140	54.55125	-5.961083
Barnett Demesne (Saplings)	BS-01	54.551876	-5.960802
	BS-02	54.551827	-5.960557
	BS-03	54.551872	-5.960586
	BS-04	54.551873	-5.960601
	BS-05	54.551788	-5.960915
	BS-06	54.551726	-5.961405
	BS-07	54.55178	-5.961518
	BS-08	54.55178	-5.961518
	BS-09	54.551844	-5.961592
	BS-10	54.551772	-5.961549
	BS-11	54.551663	-5.961477
	BS-12	54.551849	-5.960773

Table S1 (continued)

Location	Sample	Lat	Long
Barnett Demesne (Saplings)	BS-13	54.551914	-5.960383
	BS-14	54.551716	-5.961428
	BS-15	54.551929	-5.960212
	BS-16	54.552011	-5.95912
	BS-18	54.55185	-5.960881
	BS-19	54.55194	-5.960335
	BS-20	54.551817	-5.961037
	BS-21	54.551844	-5.961051
	BS-22	54.551922	-5.960877
	BS-23	54.55194	-5.960892
	BS-24	54.551922	-5.960893
	BS-26	54.551835	-5.961021
	BS-27	54.551862	-5.961035
	BS-28	54.551879	-5.961003
	BS-29	54.551869	-5.960926
	BS-30	54.55177	-5.960916
	BS-31	54.551781	-5.961054
	BS-32	54.551798	-5.960961
	BS-33	54.551836	-5.961082
	BS-34	54.551817	-5.961037
	BS-35	54.551781	-5.961023
	BS-36	54.551772	-5.961039
	BS-37	54.55179	-5.961579
	BS-38	54.55177	-5.9609
	BS-39	54.551615	-5.96131
	BS-40	54.551642	-5.961308
	BS-41	54.551641	-5.961278
	BS-42	54.551794	-5.960729
	BS-43	54.552073	-5.95971
	BS-44	54.55181	-5.96062
	BS-45	54.55181	-5.960635
	BS-46	54.552033	-5.95999
	BS-47	54.551836	-5.960572
	BS-48	54.551897	-5.96043
	BS-49	54.551913	-5.960321
	BS-50	54.551698	-5.961445
	BS-51	54.551921	-5.960831
	BS-52	54.551839	-5.961268
	BS-53	54.551799	-5.961038
	BS-54	54.551817	-5.961006
	BS-55	54.551842	-5.960927
	BS-56	54.551771	-5.960977
	BS-57	54.551725	-5.960902
	BS-58	54.551779	-5.9609
	BS-59	54.551598	-5.961342
	BS-60	54.551852	-5.960463
	BS-61	54.551892	-5.960693
	BS-62	54.551672	-5.960951

Table S1 (continued)

Location	Sample	Lat	Long
Barnett Demesne (Saplings)	BS-63	54.551852	-5.960417
	BS-64	54.551617	-5.96145
	BS-65	54.55165	-5.961417
	BS-66	54.55193	-5.960815
	BS-67	54.551781	-5.961054
	BS-68	54.551167	-5.96095
	BS-69	54.55095	-5.9606
	BS-70	54.5511	-5.960783
	BS-71	54.551167	-5.960933
	BS-72	54.551167	-5.9609
	BS-73	54.551167	-5.960883
	BS-74	54.551067	-5.960933
	BS-75	54.551117	-5.960967
	BS-76	54.550733	-5.9596
	BS-77	54.55065	-5.9595
	BS-79	54.550733	-5.959633
	BS-80	54.5509	-5.960667
	BS-81	54.550717	-5.959633
	BS-82	54.550667	-5.959633
	BS-83	54.55065	-5.959667
	BS-84	54.5506	-5.959483
	BS-85	54.550567	-5.95955
	BS-86	54.550783	-5.960283
	BS-87	54.550883	-5.960683
	BS-88	54.551317	-5.961117
	BS-89	54.55135	-5.961167
	BS-90	54.551217	-5.961017
	BS-91	54.551333	-5.9611
	BS-92	54.551067	-5.960783
	BS-93	54.550967	-5.960717
	BS-94	54.550917	-5.960667
	BS-95	54.551117	-5.960967
	BS-96	54.550883	-5.960683
Cregagh Glen (Adults)	CA-01	54.56205	-5.88915
	CA-02	54.56205	-5.88905
	CA-03	54.56435	-5.88995
	CA-04	54.564267	-5.88978
	CA-05	54.563717	-5.8897
	CA-06	54.56515	-5.889833
	CA-07	54.563533	-5.889533
	CA-08	54.5632833	-5.88945
	CA-09	54.563033	-5.88941667
	CA-10	54.562333	-5.88933
	CA-11	54.564633	-5.8897167
	CA-12	54.564167	-5.8899667
	CA-13	54.565033	-5.8899167
	CA-14	54.5620833	-5.88905

Table S1 (continued)

Location	Sample	Lat	Long
Cregagh Glen (Adults)	CA-15	54.5637	-5.8896
	CA-16	54.5644833	-5.8897
	CA-17	54.5621667	-5888733
	CA-18	54.5632167	-5.889433
	CA-19	54.5644167	-5.8897167
	CA-20	54.563733	-5.8898
	CA-21	54.5640833	-5.8898
	CA-22	54.5637667	-5.8895667
	CA-23	54.564133	-5.8894667
	CA-24	54.5678	-5.8914167
	CA-25	54.5666	-5.8909
	CA-26	54.5616	-5.8887333
	CA-27	54.5665833	-5.8910333
	CA-28	54.5665833	-5.89115
	CA-29	54.566633	-5.89115
	CA-30	54.565933	-5.8905833
	CA-31	54.5655833	-5.890416
	CA-32	54.567233	-5.89115
	CA-33	54.5668667	-5.8910167
	CA-34	54.562068	-5.888221
	CA-35	54.560998	-5.88841
	CA-36	54.5617167	-5.8885
	CA-37	54.56395	-5.8894167
	CA-38	54.5638	-5.88955
	CA-39	54.5649197	-5.88985
	CA-40	54.5641167	-5.889333
	CA-41	54.5663167	-5.890783
	CA-42	54.565033	-5.889797
	CA-43	54.565136	-5.889807
	CA-44	54.565233	-5.889806
Cregagh Glen (Saplings)	CS-01	54.565041	-5.889909
	CS-02	54.565037	-5.889909
	CS-03	54.565042	-5.889898
	CS-04	54.565037	-5.889885
	CS-05	54.565044	-5.889906
	CS-06	54.565044	-5.889908
	CS-07	54.565031	-5.889896
	CS-08	54.565033	-5.889889
	CS-10	54.565033	-5.889883
	CS-11	54.5643	-5.8898
	CS-12	54.565046	-5.889922
	CS-13	54.56429	-5.889808
	CS-14	54.565036	-5.889877
	CS-15	54.56429	-5.88979
	CS-16	54.564968	-5.890064
	CS-17	54.565041	-5.889916
	CS-18	54.565043	-5.889904

Table S1 (continued)

Location	Sample	Lat	Long
Cregagh Glen (Saplings)	CS-19	54.565045	-5.889924
	CS-20	54.565024	-5.889896
	CS-21	54.56502	-5.88989
	CS-22	54.565033	-5.8899
	CS-23	54.565033	-5.889867
	CS-24	54.565033	-5.889933
	CS-25	54.564	-5.88955
	CS-26	54.565	-5.8899167
	CS-27	54.5642	-5.88985
	CS-28	54.564	-5.88955
	CS-29	54.564	-5.889533
	CS-30	54.564	-5.8895667
	CS-31	54.564	-5.88955
	CS-32	54.56503	-5.8899
	CS-33	54.564	-5.88955
	CS-34	54.564	-5.889533
	CS-35	54.5636	-5.889533
	CS-36	54.565067	-5.8899
	CS-37	54.565033	-5.8899
	CS-38	54.564217	-5.88975
	CS-39	54.564	-5.88955
	CS-40	54.5637167	-5.8897

Table S2 Chloroplast microsatellite loci analyzed in this study.

Multiplex	Locus	Primers*	Alleles (bp)
1	AF528042.1	ACGAGCCAAAGTTCTAGCACAA	181
		GCCGGTTCGGGCTGATTTAT	
	AM933080.1	ACATTCCTCCGCTTTCATTCCT	125,127,128,129
		TCTTCCTGCCACCTTTCCCA	
	AF225238	GGGGGTAAAGACCACTCAATAAATGAA	265
		TCCTCGTACGGCTCGAGAAA	
2	AF528042.2	ATGGATGGGGTGGGGTATTAGT	224
		CTCAAATCATATCAGAGGGGTTTGC	
	JN590973	AGATAAAGGAAGGGGTCGAACG	131,132
		CAGGCCAGGCCATCAGAATAA	
	AY911655	ACAGGAATCTTTCACAAACTTCCCA	270,271
		CGAATTCCGCATATTTTCACATCTAGG	
3	AF528042.3	GCTGGTTGCTTTTTCTTTCCCA	184
		CGTCTCAACGGAGAGTTCTGAGTC	
	HM222783	CTTAGGGAAATCTCTTTCTACCG	121
		GTCAAGTCGATTCAGATTATTCCAACG	
4	AM933080.2	GGATCAAGTACGGGTTTCCGAT	122,123,124
		ACTGGAACCCTTGAATTCATTAGATACT	
	FR639483	TGACAAACCCCAGCCTAACTCT	172,173,174
		GCCTGAGCAAGTAAAGACGCTA	

* Forward tailed with CACGACGTTGTAAAACGAC; Reverse tailed with GTGTCTT

Table S3 Diversity statistics for each locus by population

Population	Locus							
	FR639485	FR646655	Femsatl-4	Femsatl-8	Femsatl-11	Femsatl-16	Femsatl-19	M230
Portaferry	$H_O = 0.552$ $H_E = 0.645$ $F_{IS} = 0.147^{NS}$	$H_O = 0.367$ $H_E = 0.461$ $F_{IS} = 0.207^{NS}$	$H_O = 0.900$ $H_E = 0.880$ $F_{IS} = -0.024^{NS}$	$H_O = 0.600$ $H_E = 0.836$ $F_{IS} = 0.286^{***}$	$H_O = 0.759$ $H_E = 0.893$ $F_{IS} = 0.153^{*}$	$H_O = 0.448$ $H_E = 0.448$ $F_{IS} = 0.000^{NS}$	$H_O = 0.897$ $H_E = 0.908$ $F_{IS} = 0.013^{NS}$	$H_O = 0.862$ $H_E = 0.926$ $F_{IS} = 0.070^{NS}$
Downpatrick	$H_O = 0.428$ $H_E = 0.442$ $F_{IS} = 0.031^{NS}$	$H_O = 0.345$ $H_E = 0.448$ $F_{IS} = 0.234^{NS}$	$H_O = 0.759$ $H_E = 0.737$ $F_{IS} = -0.028^{NS}$	$H_O = 0.483$ $H_E = 0.912$ $F_{IS} = 0.475^{***}$	$H_O = 0.897$ $H_E = 0.897$ $F_{IS} = 0.000^{NS}$	$H_O = 0.500$ $H_E = 0.590$ $F_{IS} = 0.155^{NS}$	$H_O = 0.862$ $H_E = 0.890$ $F_{IS} = 0.032^{NS}$	$H_O = 0.900$ $H_E = 0.958$ $F_{IS} = 0.062^{NS}$
Helen's Bay	$H_O = 0.536$ $H_E = 0.544$ $F_{IS} = 0.016^{NS}$	$H_O = 0.379$ $H_E = 0.468$ $F_{IS} = 0.192^{NS}$	$H_O = 0.700$ $H_E = 0.843$ $F_{IS} = 0.172^{*}$	$H_O = 0.593$ $H_E = 0.872$ $F_{IS} = 0.325^{***}$	$H_O = 0.931$ $H_E = 0.859$ $F_{IS} = -0.085^{NS}$	$H_O = 0.433$ $H_E = 0.433$ $F_{IS} = 0.000^{NS}$	$H_O = 0.793$ $H_E = 0.930$ $F_{IS} = 0.150^{**}$	$H_O = 0.884$ $H_E = 0.890$ $F_{IS} = 0.006^{NS}$
Magheramourne	$H_O = 0.621$ $H_E = 0.623$ $F_{IS} = 0.003^{NS}$	$H_O = 0.500$ $H_E = 0.497$ $F_{IS} = -0.007^{NS}$	$H_O = 0.933$ $H_E = 0.870$ $F_{IS} = -0.074^{NS}$	$H_O = 0.767$ $H_E = 0.904$ $F_{IS} = 0.152^{*}$	$H_O = 0.900$ $H_E = 0.896$ $F_{IS} = -0.004^{NS}$	$H_O = 0.633$ $H_E = 0.627$ $F_{IS} = -0.010^{NS}$	$H_O = 0.900$ $H_E = 0.905$ $F_{IS} = 0.006^{NS}$	$H_O = 1.000$ $H_E = 0.949$ $F_{IS} = -0.055^{NS}$
Glenarm Forest	$H_O = 0.759$ $H_E = 0.734$ $F_{IS} = -0.034^{NS}$	$H_O = 0.467$ $H_E = 0.494$ $F_{IS} = 0.057^{NS}$	$H_O = 1.000$ $H_E = 0.828$ $F_{IS} = -0.213^{NS}$	$H_O = 0.897$ $H_E = 0.881$ $F_{IS} = -0.018^{NS}$	$H_O = 0.867$ $H_E = 0.830$ $F_{IS} = -0.045^{NS}$	$H_O = 0.733$ $H_E = 0.596$ $F_{IS} = -0.235^{NS}$	$H_O = 0.862$ $H_E = 0.899$ $F_{IS} = 0.042^{NS}$	$H_O = 1.000$ $H_E = 0.901$ $F_{IS} = -0.112^{NS}$
Barnett Demesne	$H_O = 0.679$ $H_E = 0.521$ $F_{IS} = -0.310^{NS}$	$H_O = 0.536$ $H_E = 0.529$ $F_{IS} = -0.013^{NS}$	$H_O = 0.929$ $H_E = 0.889$ $F_{IS} = -0.045^{NS}$	$H_O = 0.759$ $H_E = 0.919$ $F_{IS} = 0.177^{*}$	$H_O = 0.793$ $H_E = 0.885$ $F_{IS} = 0.106^{NS}$	$H_O = 0.418$ $H_E = 0.497$ $F_{IS} = 0.169^{NS}$	$H_O = 0.793$ $H_E = 0.913$ $F_{IS} = 0.133^{*}$	$H_O = 0.966$ $H_E = 0.917$ $F_{IS} = -0.054^{NS}$
Trassey Road	$H_O = 0.418$ $H_E = 0.492$ $F_{IS} = 0.162^{NS}$	$H_O = 0.345$ $H_E = 0.328$ $F_{IS} = -0.051^{NS}$	$H_O = 0.897$ $H_E = 0.898$ $F_{IS} = 0.001^{NS}$	$H_O = 0.750$ $H_E = 0.879$ $F_{IS} = 0.149^{*}$	$H_O = 0.893$ $H_E = 0.917$ $F_{IS} = 0.027^{NS}$	$H_O = 0.310$ $H_E = 0.337$ $F_{IS} = 0.080^{NS}$	$H_O = 0.759$ $H_E = 0.901$ $F_{IS} = 0.160^{*}$	$H_O = 0.931$ $H_E = 0.949$ $F_{IS} = 0.019^{NS}$

Table S3 (cont.)

Population	Locus							
	FR639485	FR646655	Femsatl-4	Femsatl-8	Femsatl-11	Femsatl-16	Femsatl-19	M230
Dromara	H_O = 0.607 H_E = 0.609 F_{IS} = 0.003 ^{NS}	H_O = 0.429 H_E = 0.577 F_{IS} = 0.260*	H_O = 0.862 H_E = 0.873 F_{IS} = 0.013^{NS}	H_O = 0.793 H_E = 0.938 F_{IS} = 0.157**	H_O = 0.824 H_E = 0.897 F_{IS} = 0.084^{NS}	H_O = 0.536 H_E = 0.494 F_{IS} = -0.087^{NS}	H_O = 0.828 H_E = 0.913 F_{IS} = 0.096^{NS}	H_O = 0.897 H_E = 0.954 F_{IS} = 0.061^{NS}
Hillsborough	H_O = 0.433 H_E = 0.586 F_{IS} = 0.264^{NS}	H_O = 0.700 H_E = 0.677 F_{IS} = -0.034^{NS}	H_O = 0.679 H_E = 0.877 F_{IS} = 0.229**	H_O = 0.600 H_E = 0.929 F_{IS} = 0.358***	H_O = 0.900 H_E = 0.849 F_{IS} = -0.06^{NS}	H_O = 0.400 H_E = 0.458 F_{IS} = 0.128^{NS}	H_O = 0.769 H_E = 0.911 F_{IS} = 0.170**	H_O = 0.967 H_E = 0.949 F_{IS} = -0.019^{NS}
Glenariff Forest	H_O = 0.655 H_E = 0.620 F_{IS} = -0.058^{NS}	H_O = 0.400 H_E = 0.513 F_{IS} = 0.223*	H_O = 0.893 H_E = 0.925 F_{IS} = 0.035^{NS}	H_O = 0.759 H_E = 0.895 F_{IS} = 0.155*	H_O = 0.900 H_E = 0.889 F_{IS} = -0.012^{NS}	H_O = 0.517 H_E = 0.514 F_{IS} = -0.006^{NS}	H_O = 0.759 H_E = 0.887 F_{IS} = 0.147*	H_O = 0.933 H_E = 0.954 F_{IS} = 0.022^{NS}
Rostrevor	H_O = 0.571 H_E = 0.692 F_{IS} = 0.176^{NS}	H_O = 0.464 H_E = 0.384 F_{IS} = -0.215^{NS}	H_O = 0.893 H_E = 0.863 F_{IS} = -0.035^{NS}	H_O = 0.750 H_E = 0.865 F_{IS} = 0.135^{NS}	H_O = 0.964 H_E = 0.918 F_{IS} = -0.052^{NS}	H_O = 0.500 H_E = 0.444 F_{IS} = -0.130^{NS}	H_O = 0.786 H_E = 0.873 F_{IS} = 0.102^{NS}	H_O = 0.964 H_E = 0.952 F_{IS} = -0.013^{NS}
Ballycastle Forest	H_O = 0.533 H_E = 0.576 F_{IS} = 0.076^{NS}	H_O = 0.567 H_E = 0.485 F_{IS} = -0.171^{NS}	H_O = 0.933 H_E = 0.920 F_{IS} = -0.014^{NS}	H_O = 0.759 H_E = 0.874 F_{IS} = 0.134*	H_O = 0.963 H_E = 0.893 F_{IS} = -0.080^{NS}	H_O = 0.660 H_E = 0.544 F_{IS} = -0.105^{NS}	H_O = 0.900 H_E = 0.876 F_{IS} = -0.028^{NS}	H_O = 0.786 H_E = 0.892 F_{IS} = 0.121^{NS}
Randalstown	H_O = 0.733 H_E = 0.669 F_{IS} = -0.097^{NS}	H_O = 0.552 H_E = 0.590 F_{IS} = 0.067^{NS}	H_O = 0.900 H_E = 0.912 F_{IS} = 0.014^{NS}	H_O = 0.733 H_E = 0.928 F_{IS} = 0.212***	H_O = 0.833 H_E = 0.911 F_{IS} = 0.086^{NS}	H_O = 0.500 H_E = 0.611 F_{IS} = 0.185^{NS}	H_O = 0.633 H_E = 0.898 F_{IS} = 0.299***	H_O = 0.967 H_E = 0.948 F_{IS} = -0.020^{NS}
Portglenone	H_O = 0.833 H_E = 0.758 F_{IS} = -0.101^{NS}	H_O = 0.500 H_E = 0.544 F_{IS} = 0.081^{NS}	H_O = 0.862 H_E = 0.858 F_{IS} = -0.004^{NS}	H_O = 0.633 H_E = 0.918 F_{IS} = 0.313***	H_O = 0.867 H_E = 0.891 F_{IS} = 0.028^{NS}	H_O = 0.586 H_E = 0.620 F_{IS} = 0.056^{NS}	H_O = 0.800 H_E = 0.914 F_{IS} = 0.127*	H_O = 0.828 H_E = 0.910 F_{IS} = 0.093^{NS}

Table S3 (cont.)

Population	Locus							
	FR639485	FR646655	Femsatl-4	Femsatl-8	Femsatl-11	Femsatl-16	Femsatl-19	M230
Gosford Park	H_O = 0.552 H_E = 0.606 F_{IS} = 0.090^{NS}	H_O = 0.517 H_E = 0.506 F_{IS} = -0.022^{NS}	H_O = 0.867 H_E = 0.912 F_{IS} = 0.051^{NS}	H_O = 0.633 H_E = 0.895 F_{IS} = 0.296***	H_O = 1.000 H_E = 0.913 F_{IS} = -0.097^{NS}	H_O = 0.567 H_E = 0.489 F_{IS} = -0.163^{NS}	H_O = 0.800 H_E = 0.894 F_{IS} = 0.107^{NS}	H_O = 0.967 H_E = 0.951 F_{IS} = -0.016^{NS}
Ballymoney	H_O = 0.655 H_E = 0.630 F_{IS} = -0.040^{NS}	H_O = 0.367 H_E = 0.472 F_{IS} = 0.226*	H_O = 0.750 H_E = 0.886 F_{IS} = 0.156*	H_O = 0.690 H_E = 0.913 F_{IS} = 0.248***	H_O = 0.815 H_E = 0.853 F_{IS} = 0.045^{NS}	H_O = 0.633 H_E = 0.616 F_{IS} = -0.028^{NS}	H_O = 0.733 H_E = 0.873 F_{IS} = 0.163*	H_O = 0.933 H_E = 0.926 F_{IS} = -0.008^{NS}
Peatlands Park	H_O = 0.690 H_E = 0.626 F_{IS} = -0.105^{NS}	H_O = 0.345 H_E = 0.388 F_{IS} = 0.113^{NS}	H_O = 0.655 H_E = 0.814 F_{IS} = 0.198*	H_O = 0.536 H_E = 0.886 F_{IS} = 0.400***	H_O = 0.828 H_E = 0.840 F_{IS} = 0.015^{NS}	H_O = 0.464 H_E = 0.430 F_{IS} = -0.082^{NS}	H_O = 0.862 H_E = 0.909 F_{IS} = 0.052^{NS}	H_O = 0.964 H_E = 0.946 F_{IS} = -0.020^{NS}
Carndaisy Woods	H_O = 0.552 H_E = 0.657 F_{IS} = 0.163^{NS}	H_O = 0.367 H_E = 0.457 F_{IS} = 0.199^{NS}	H_O = 0.800 H_E = 0.891 F_{IS} = 0.104^{NS}	H_O = 0.767 H_E = 0.920 F_{IS} = 0.169**	H_O = 0.767 H_E = 0.864 F_{IS} = 0.115^{NS}	H_O = 0.667 H_E = 0.550 F_{IS} = -0.216^{NS}	H_O = 0.800 H_E = 0.924 F_{IS} = 0.136*	H_O = 0.800 H_E = 0.925 F_{IS} = 0.137*
Downhill	H_O = 0.621 H_E = 0.719 F_{IS} = 0.139^{NS}	H_O = 0.414 H_E = 0.402 F_{IS} = -0.029^{NS}	H_O = 0.655 H_E = 0.910 F_{IS} = 0.284***	H_O = 0.556 H_E = 0.929 F_{IS} = 0.406***	H_O = 0.897 H_E = 0.905 F_{IS} = 0.010^{NS}	H_O = 0.517 H_E = 0.431 F_{IS} = -0.203^{NS}	H_O = 0.621 H_E = 0.873 F_{IS} = 0.293***	H_O = 0.966 H_E = 0.951 F_{IS} = -0.016^{NS}
Drum Manor	H_O = 0.536 H_E = 0.622 F_{IS} = 0.141^{NS}	H_O = 0.467 H_E = 0.502 F_{IS} = 0.071^{NS}	H_O = 0.964 H_E = 0.913 F_{IS} = -0.057^{NS}	H_O = 0.867 H_E = 0.906 F_{IS} = 0.044^{NS}	H_O = 0.833 H_E = 0.851 F_{IS} = 0.021^{NS}	H_O = 0.586 H_E = 0.547 F_{IS} = -0.072^{NS}	H_O = 0.633 H_E = 0.933 F_{IS} = 0.325***	H_O = 0.929 H_E = 0.956 F_{IS} = 0.029^{NS}
Lemnagore Wood	H_O = 0.586 H_E = 0.576 F_{IS} = -0.018^{NS}	H_O = 0.414 H_E = 0.413 F_{IS} = -0.001^{NS}	H_O = 0.964 H_E = 0.818 F_{IS} = -0.183^{NS}	H_O = 0.724 H_E = 0.906 F_{IS} = 0.204**	H_O = 0.929 H_E = 0.927 F_{IS} = -0.002^{NS}	H_O = 0.250 H_E = 0.265 F_{IS} = 0.057^{NS}	H_O = 0.846 H_E = 0.916 F_{IS} = 0.077^{NS}	H_O = 1.000 H_E = 0.939 F_{IS} = -0.066^{NS}

Table S3 (cont.)

Population	Locus							
	FR639485	FR646655	Femsatl-4	Femsatl-8	Femsatl-11	Femsatl-16	Femsatl-19	M230
Roe Valley	H_O = 0.533 H_E = 0.642 F_{IS} = 0.172^{NS}	H_O = 0.533 H_E = 0.596 F_{IS} = 0.107^{NS}	H_O = 0.778 H_E = 0.882 F_{IS} = 0.120^{NS}	H_O = 0.679 H_E = 0.918 F_{IS} = 0.265***	H_O = 0.893 H_E = 0.907 F_{IS} = 0.016^{NS}	H_O = 0.400 H_E = 0.451 F_{IS} = 0.116^{NS}	H_O = 0.571 H_E = 0.873 F_{IS} = 0.350***	H_O = 0.966 H_E = 0.949 F_{IS} = -0.018^{NS}
Knockmany Forest	H_O = 0.833 H_E = 0.669 F_{IS} = -0.250^{NS}	H_O = 0.567 H_E = 0.455 F_{IS} = -0.250^{NS}	H_O = 0.933 H_E = 0.916 F_{IS} = -0.019^{NS}	H_O = 0.700 H_E = 0.918 F_{IS} = 0.240***	H_O = 0.833 H_E = 0.911 F_{IS} = 0.087^{NS}	H_O = 0.667 H_E = 0.564 F_{IS} = -0.186^{NS}	H_O = 0.900 H_E = 0.889 F_{IS} = -0.013^{NS}	H_O = 0.967 H_E = 0.933 F_{IS} = -0.036^{NS}
Slieve Beagh	H_O = 0.643 H_E = 0.599 F_{IS} = -0.075^{NS}	H_O = 0.536 H_E = 0.497 F_{IS} = -0.079^{NS}	H_O = 0.786 H_E = 0.873 F_{IS} = 0.102^{NS}	H_O = 0.750 H_E = 0.931 F_{IS} = 0.197**	H_O = 0.750 H_E = 0.818 F_{IS} = 0.085^{NS}	H_O = 0.357 H_E = 0.317 F_{IS} = -0.130^{NS}	H_O = 0.679 H_E = 0.898 F_{IS} = 0.248***	H_O = 0.857 H_E = 0.932 F_{IS} = 0.082^{NS}
Stranbane Glen	H_O = 0.483 H_E = 0.606 F_{IS} = 0.206^{NS}	H_O = 0.433 H_E = 0.553 F_{IS} = 0.219^{NS}	H_O = 0.900 H_E = 0.888 F_{IS} = -0.014^{NS}	H_O = 0.793 H_E = 0.922 F_{IS} = 0.142*	H_O = 0.862 H_E = 0.909 F_{IS} = 0.053^{NS}	H_O = 0.467 H_E = 0.448 F_{IS} = -0.042^{NS}	H_O = 0.833 H_E = 0.904 F_{IS} = 0.079^{NS}	H_O = 0.897 H_E = 0.951 F_{IS} = 0.058^{NS}
Crom	H_O = 0.552 H_E = 0.621 F_{IS} = 0.113^{NS}	H_O = 0.500 H_E = 0.460 F_{IS} = -0.088^{NS}	H_O = 0.833 H_E = 0.891 F_{IS} = 0.066^{NS}	H_O = 0.679 H_E = 0.939 F_{IS} = 0.281***	H_O = 0.724 H_E = 0.836 F_{IS} = 0.137^{NS}	H_O = 0.400 H_E = 0.456 F_{IS} = 0.125^{NS}	H_O = 0.867 H_E = 0.897 F_{IS} = 0.034^{NS}	H_O = 0.800 H_E = 0.941 F_{IS} = 0.152**
Knockninny Hill	H_O = 0.607 H_E = 0.642 F_{IS} = 0.055^{NS}	H_O = 0.393 H_E = 0.384 F_{IS} = -0.024^{NS}	H_O = 1.000 H_E = 0.929 F_{IS} = -0.078^{NS}	H_O = 0.464 H_E = 0.919 F_{IS} = 0.500***	H_O = 0.929 H_E = 0.893 F_{IS} = -0.041^{NS}	H_O = 0.429 H_E = 0.525 F_{IS} = 0.187^{NS}	H_O = 0.821 H_E = 0.907 F_{IS} = 0.096^{NS}	H_O = 0.889 H_E = 0.957 F_{IS} = 0.072^{NS}
Sloughan Glen	H_O = 0.690 H_E = 0.584 F_{IS} = -0.184^{NS}	H_O = 0.483 H_E = 0.451 F_{IS} = -0.073^{NS}	H_O = 0.897 H_E = 0.874 F_{IS} = -0.027^{NS}	H_O = 0.655 H_E = 0.909 F_{IS} = 0.283***	H_O = 0.862 H_E = 0.855 F_{IS} = -0.008^{NS}	H_O = 0.414 H_E = 0.453 F_{IS} = 0.088^{NS}	H_O = 0.714 H_E = 0.898 F_{IS} = 0.208**	H_O = 0.862 H_E = 0.935 F_{IS} = 0.080^{NS}

Table S3 (cont.)

Population	Locus							
	FR639485	FR646655	Femsatl-4	Femsatl-8	Femsatl-11	Femsatl-16	Femsatl-19	M230
Castle Hill	H_O = 0.467 H_E = 0.704 F_{IS} = 0.341**	H_O = 0.200 H_E = 0.367 F_{IS} = 0.461**	H_O = 0.800 H_E = 0.866 F_{IS} = 0.078^{NS}	H_O = 0.633 H_E = 0.903 F_{IS} = 0.302***	H_O = 0.833 H_E =0.877 F_{IS} = 0.051^{NS}	H_O = 0.467 H_E = 0.471 F_{IS} = 0.009^{NS}	H_O = 0.867 H_E = 0.911 F_{IS} = 0.049^{NS}	H_O = 0.828 H_E = 0.955 F_{IS} = 0.135**
Ely Lodge	H_O = 0.621 H_E = 0.650 F_{IS} = 0.045^{NS}	H_O = 0.483 H_E = 0.550 F_{IS} = 0.124^{NS}	H_O = 0.900 H_E = 0.912 F_{IS} = 0.013^{NS}	H_O = 0.724 H_E = 0.939 F_{IS} = 0.232***	H_O = 0.800 H_E = 0.911 F_{IS} = 0.124^{NS}	H_O = 0.433 H_E = 0.419 F_{IS} = -0.036^{NS}	H_O = 0.900 H_E = 0.904 F_{IS} = 0.004^{NS}	H_O = 0.964 H_E = 0.920 F_{IS} = -0.049^{NS}
Killeter Forest	H_O = 0.793 H_E = 0.663 F_{IS} = -0.200^{NS}	H_O = 0.467 H_E = 0.445 F_{IS} = -0.050^{NS}	H_O = 0.767 H_E = 0.849 F_{IS} = 0.098^{NS}	H_O = 0.857 H_E = 0.924 F_{IS} = 0.074^{NS}	H_O = 0.862 H_E = 0.903 F_{IS} = 0.046^{NS}	H_O = 0.567 H_E = 0.547 F_{IS} = -0.036^{NS}	H_O = 0.724 H_E = 0.935 F_{IS} = 0.228***	H_O = 0.867 H_E = 0.964 F_{IS} = 0.102*
Marble Arch	H_O = 0.522 H_E = 0.610 F_{IS} = 0.147^{NS}	H_O = 0.259 H_E = 0.349 F_{IS} = 0.262^{NS}	H_O = 0.893 H_E = 0.888 F_{IS} = -0.005^{NS}	H_O = 0.808 H_E = 0.937 F_{IS} = 0.140*	H_O = 0.857 H_E = 0.866 F_{IS} = 0.010^{NS}	H_O = 0.571 H_E = 0.598 F_{IS} = 0.045^{NS}	H_O = 0.889 H_E = 0.918 F_{IS} = 0.032^{NS}	H_O = 0.885 H_E = 0.941 F_{IS} = 0.061^{NS}
Castle Caldwell	H_O = 0.724 H_E = 0.665 F_{IS} = -0.091^{NS}	H_O = 0.172 H_E = 0.226 F_{IS} = 0.241^{NS}	H_O = 0.897 H_E = 0.836 F_{IS} = -0.074^{NS}	H_O = 0.533 H_E = 0.936 F_{IS} = 0.434***	H_O = 0.867 H_E = 0.895 F_{IS} = 0.033^{NS}	H_O = 0.533 H_E = 0.517 F_{IS} = -0.032^{NS}	H_O = 0.900 H_E = 0.920 F_{IS} = 0.022^{NS}	H_O = 0.867 H_E = 0.937 F_{IS} = 0.077^{NS}
Glenasmole Valley	H_O = 0.821 H_E = 0.738 F_{IS} = -0.116^{NS}	H_O = 0.500 H_E = 0.466 F_{IS} = -0.075^{NS}	H_O = 0.926 H_E = 0.897 F_{IS} = -0.033^{NS}	H_O = 0.750 H_E = 0.894 F_{IS} = 0.163*	H_O = 0.821 H_E = 0.894 F_{IS} = 0.082^{NS}	H_O = 0.500 H_E = 0.477 F_{IS} = -0.050^{NS}	H_O = 0.893 H_E = 0.898 F_{IS} = 0.006^{NS}	H_O = 0.885 H_E = 0.945 F_{IS} = 0.065^{NS}
Knocknamallavoge	H_O = 0.828 H_E = 0.638 F_{IS} = -0.304^{NS}	H_O = 0.552 H_E = 0.757 F_{IS} = 0.274*	H_O = 0.724 H_E = 0.822 F_{IS} = 0.121^{NS}	H_O = 0.724 H_E = 0.910 F_{IS} = 0.208**	H_O = 0.759 H_E = 0.866 F_{IS} = 0.126^{NS}	H_O = 0.621 H_E = 0.614 F_{IS} = -0.011^{NS}	H_O = 0.828 H_E = 0.918 F_{IS} = 0.100^{NS}	H_O = 0.931 H_E = 0.891 F_{IS} = -0.046^{NS}

Table S3 (cont.)

Population	Locus							
	FR639485	FR646655	Femsatl-4	Femsatl-8	Femsatl-11	Femsatl-16	Femsatl-19	M230
Letterfrack	$H_O = 0.423$ $H_E = 0.659$ $F_{IS} = 0.363$**	$H_O = 0.385$ $H_E = 0.465$ $F_{IS} = 0.176^{NS}$	$H_O = 0.800$ $H_E = 0.914$ $F_{IS} = 0.127$*	$H_O = 0.640$ $H_E = 0.825$ $F_{IS} = 0.228$**	$H_O = 0.885$ $H_E = 0.902$ $F_{IS} = 0.020^{NS}$	$H_O = 0.391$ $H_E = 0.580$ $F_{IS} = 0.330$*	$H_O = 0.818$ $H_E = 0.909$ $F_{IS} = 0.102^{NS}$	$H_O = 0.808$ $H_E = 0.911$ $F_{IS} = 0.115^{NS}$

Figure Legends

Fig. 1 Locations of sites sampled in this study. Numbers correspond to those in Table 1.

Fig. 2. Two woodland sites sampled at (a) Belfast, Northern Ireland (insert) showing tree cover from orthophotographs of (b) Barnett Demesne and c) Cregagh Glen. Adult trees that were sampled are shown as red dots and saplings as blue dots; surrounding trees, hedgerows and woodlands are clearly visible. North is aligned with the top of the page. Image © 07/08/2006 DigitalGlobe, Google Earth.

Fig. 3. Mantel test for isolation-by-distance (IBD) between populations including (above) and excluding (below) the three Republic of Ireland populations.

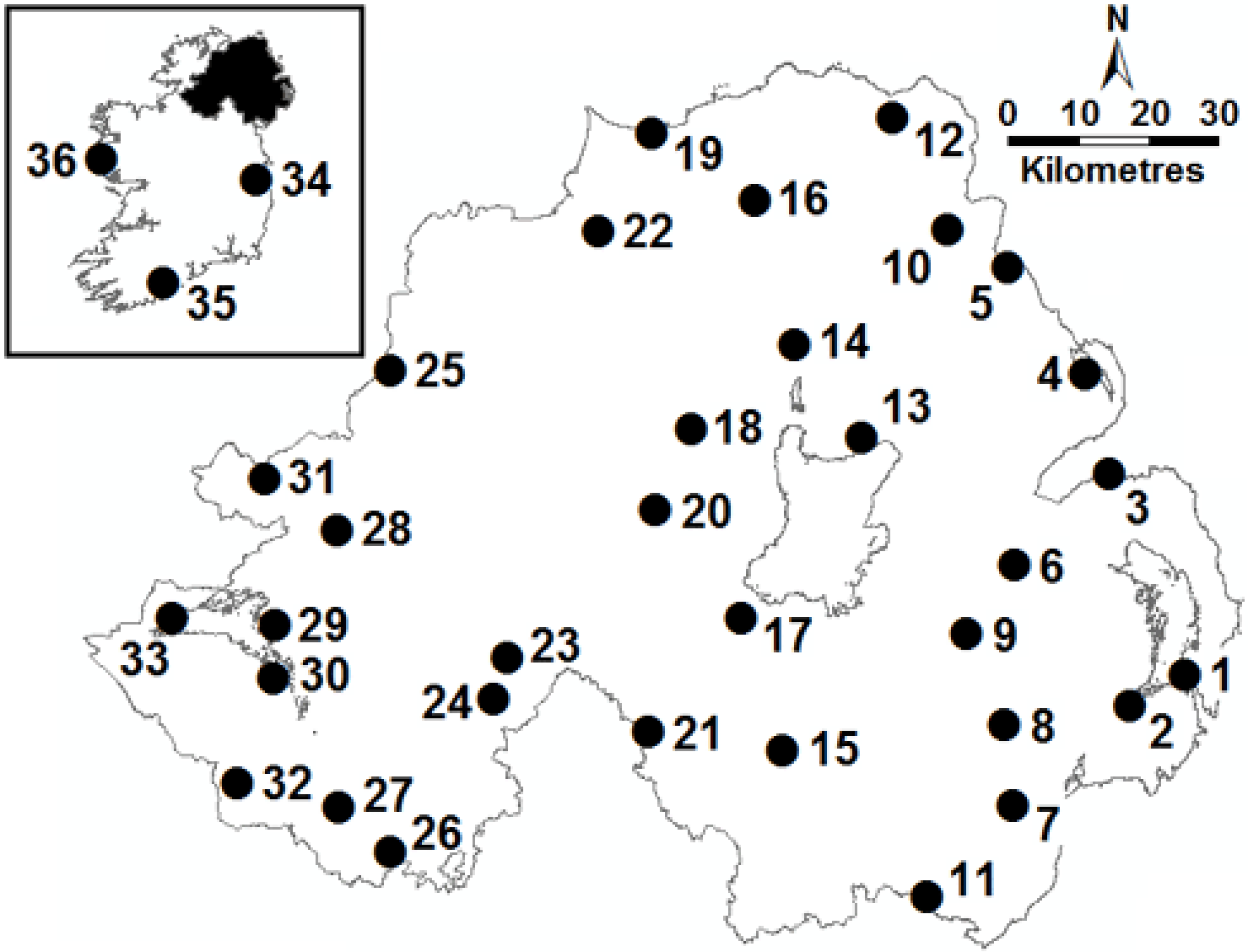
Fig. 4. Summary of identified seed dispersal events in Barnett Demesne. (a) Histogram showing dispersal distances in 10 m classes. Black shading indicates assignment of a maternal plant from a parent-pair. Grey shading shows assignment of a maternal plant to a single identified parent. (b) Distance and direction of identified dispersal events. Broken arrows represent dispersal distances of greater than 50 m (values given in parentheses).

Fig. S1 Network showing relationships between the eight cpSSR haplotypes observed. Each mutation is shown by a dash, with the locus and allele size change indicated. An alternative homoplasious linkage between haplotypes H2 and H7 is indicated by the dashed line.

Fig. S2 Correlograms of autocorrelation coefficient (θ; y-axis) plotted against distance (x-axis). 95% confidence intervals are indicated by dashed red lines. Note that in some

correlograms, the first two distance intervals (0 – 50 m and 50 – 100 m) may be at a different scale to subsequent intervals.

Fig. S3 Example of large allele dropout in consecutive individuals at locus M230. Note that in both cases, the large allele has not been called by the genotyping software.


N
0 10 20 30
Kilometres
36
34
35
19
12
16
22
10
5
14
25
4
18
13
31
3
20
28
6
29
17
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23
1
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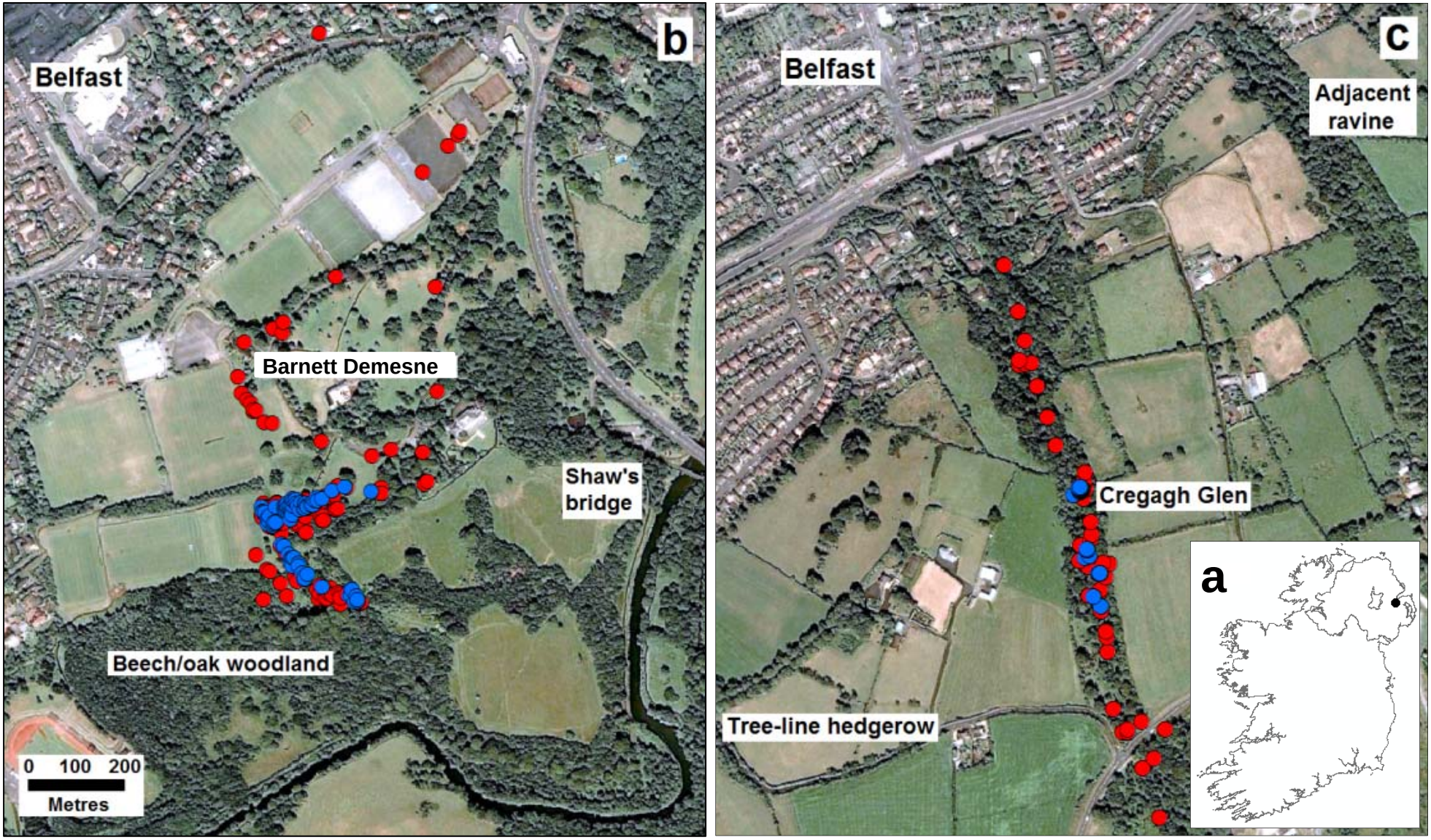
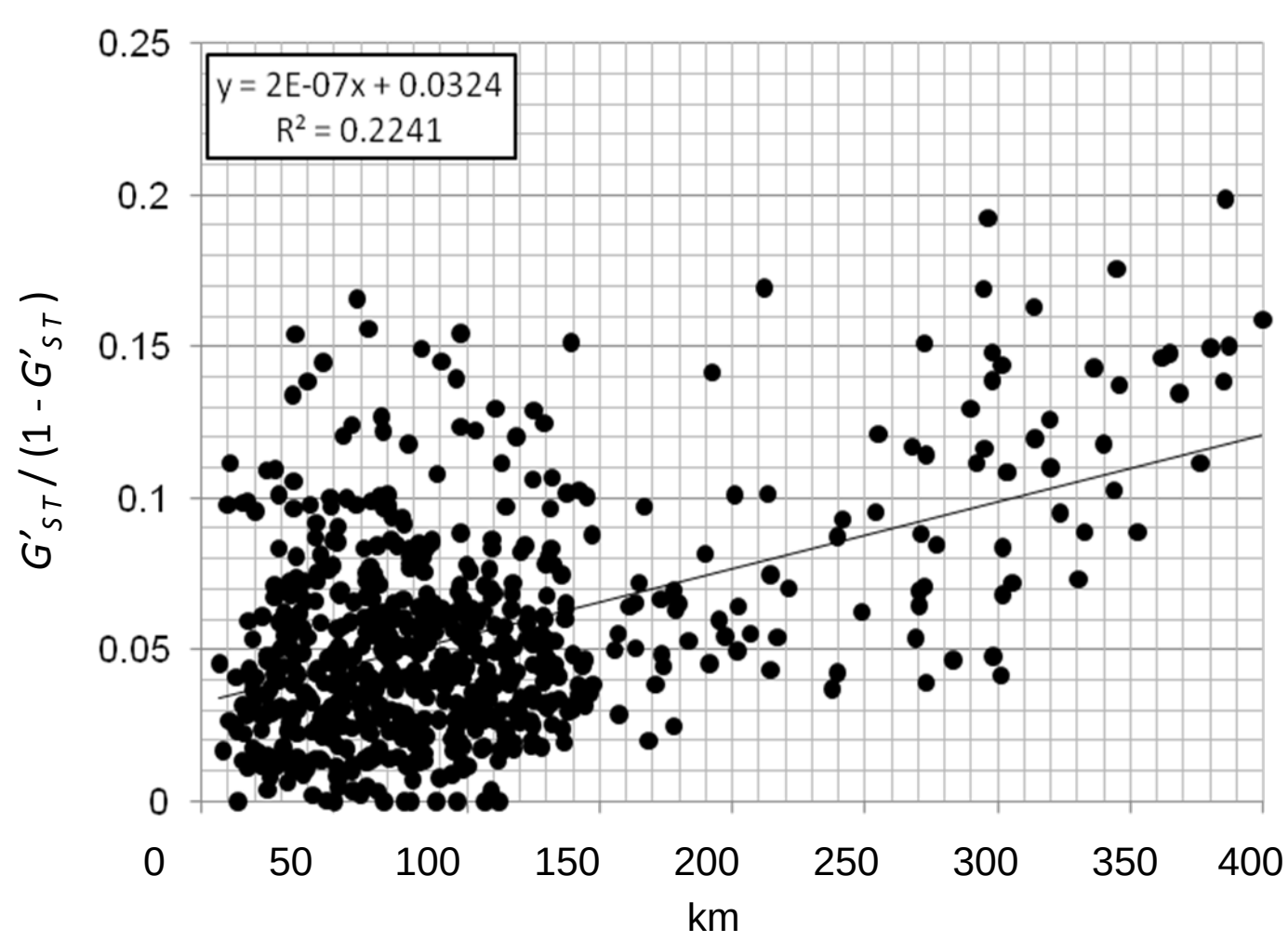
15
8
2
32
27
7
26
11


b
Belfast
Barnett Demesne
Shaw's bridge
Beech/oak woodland
0 100 200
Metres
c
Belfast
Adjacent ravine
Cregagh Glen
Tree-line hedgerow
a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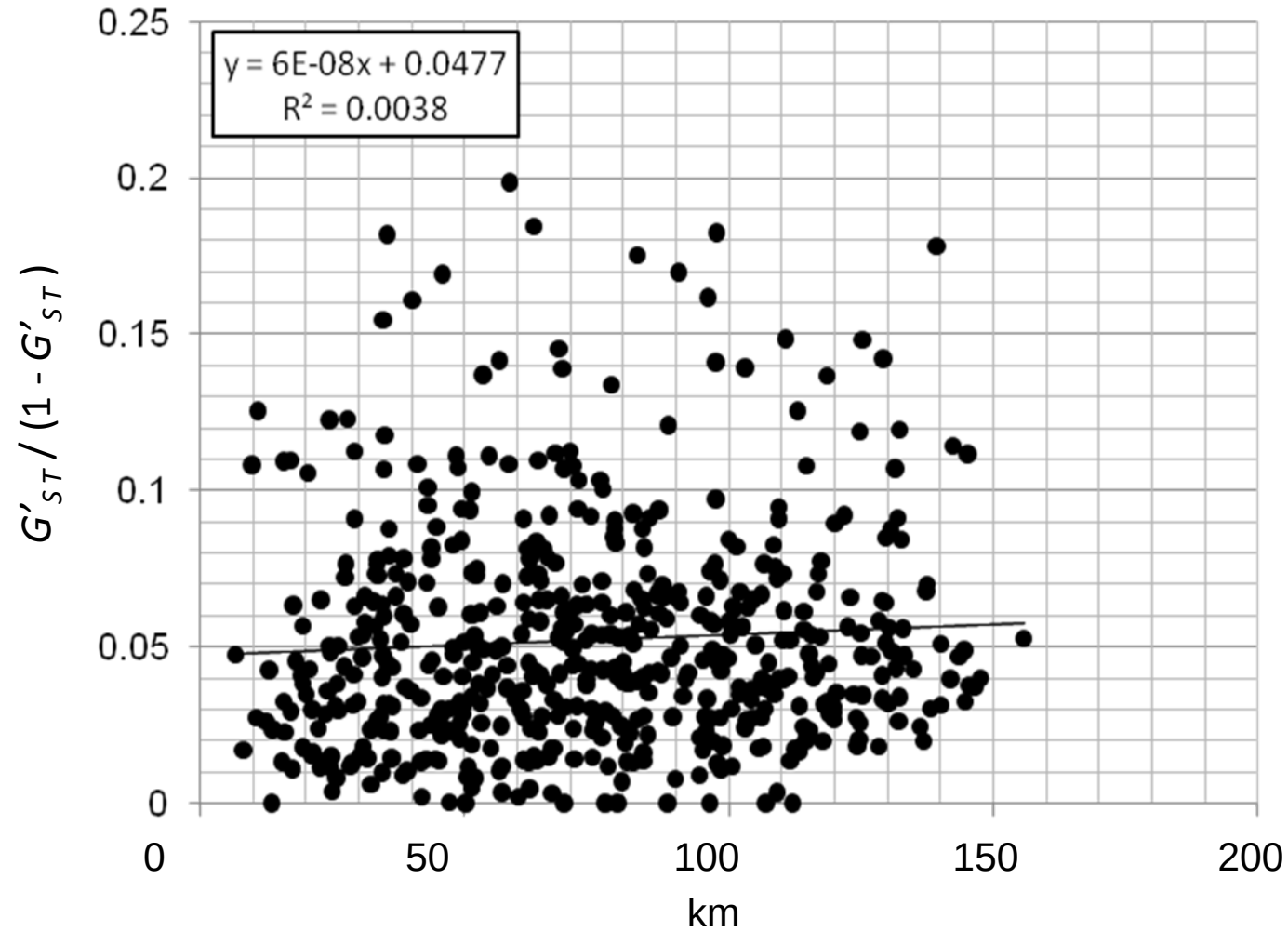
R² = 0.2241
0.25
0.2
0.15
0.1
0.05
0
0
50
100
150
200
250
300
350
400
km
G′ST / (1 - G′ST)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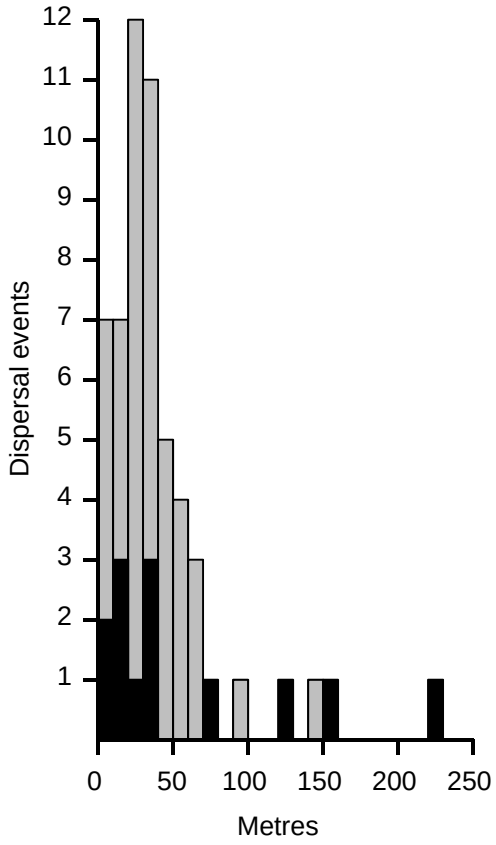
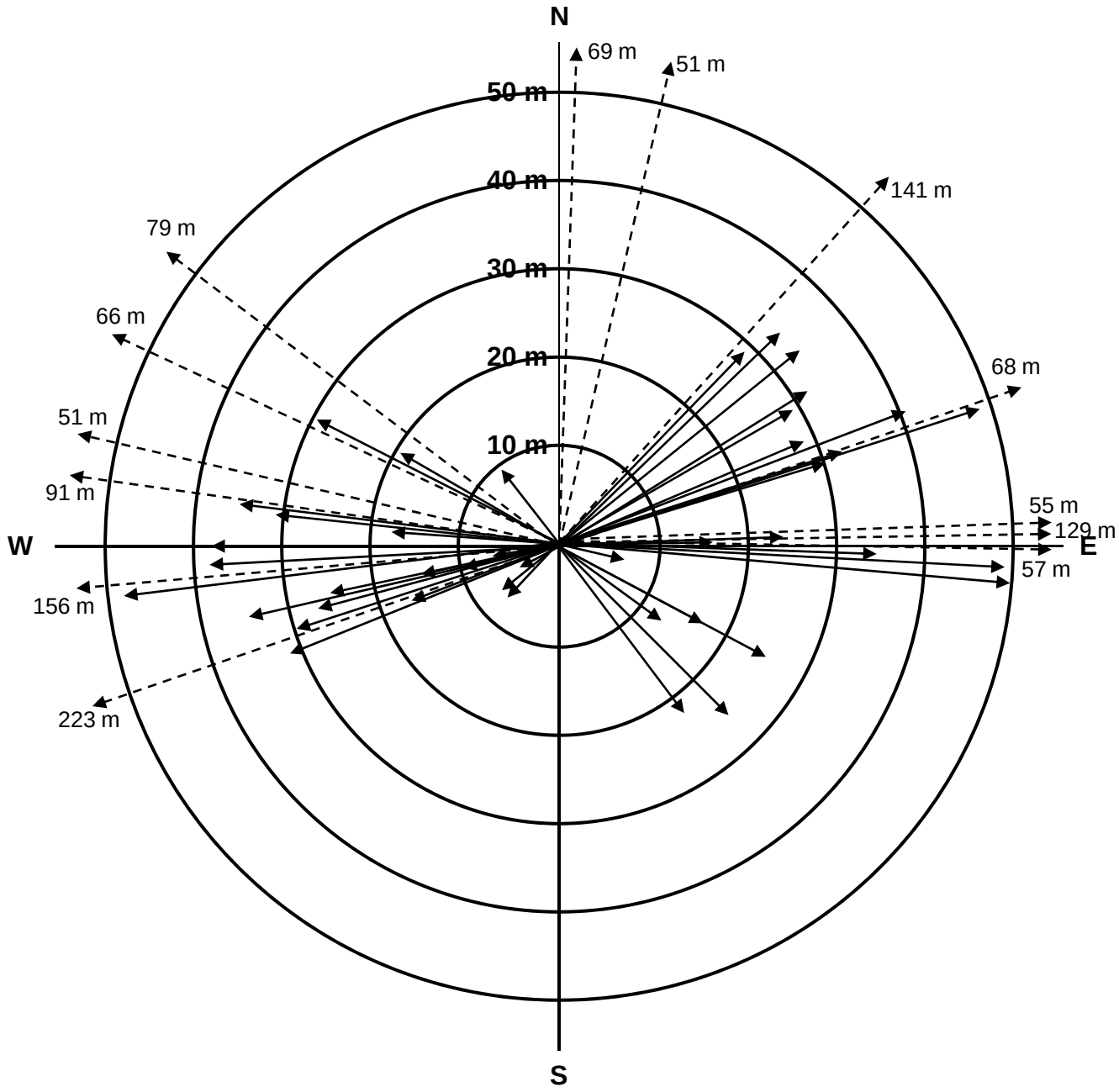
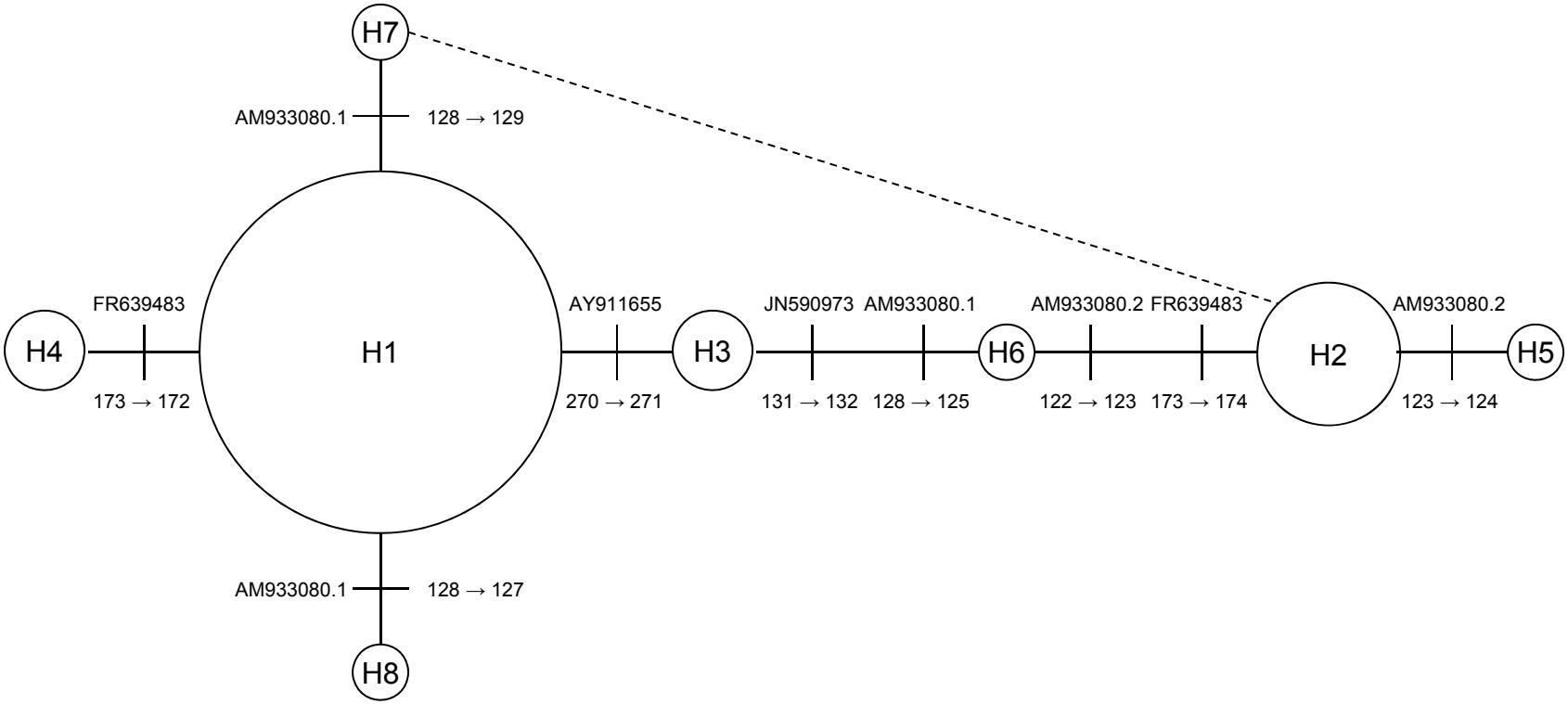
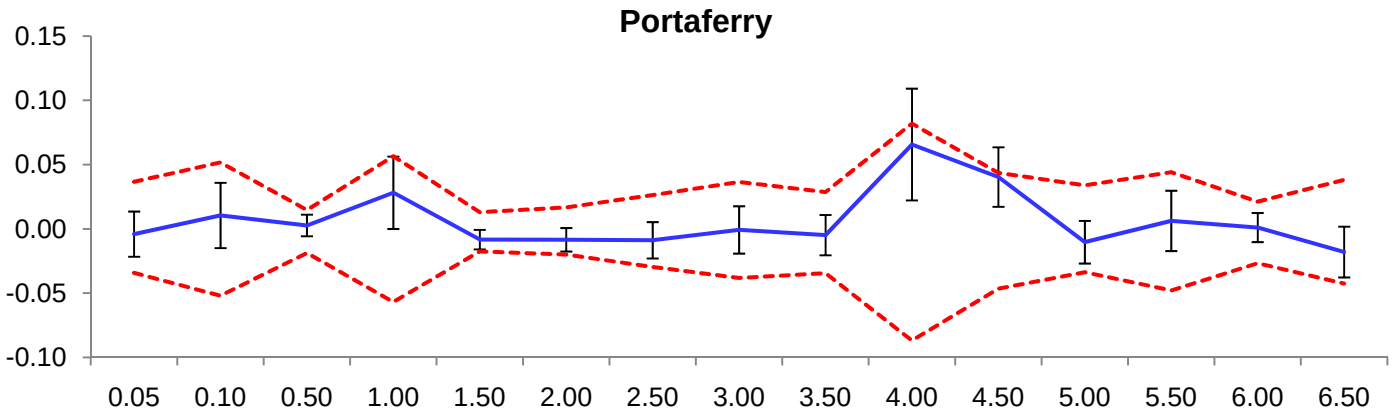
0.2
0.15
0.1
0.05
0
0
50
100
150
200
km
G′ST / (1 - G′ST)


Dispersal events
12
11
10
9
8
7
6
5
4
3
2
1
0
50
100
150
200
250
Metres


N
S
W
E
50 m
40 m
30 m
20 m
10 m
69 m
51 m
141 m
79 m
66 m
68 m
51 m
91 m
55 m
129 m
57 m
156 m
223 m


H7
AM933080.1
128 → 129
FR639483
H4
173 → 172
H1
AY911655
270 → 271
H3
JN590973
131 → 132
AM933080.1
128 → 125
H6
AM933080.2
122 → 123
FR639483
173 → 174
H2
AM933080.2
123 → 124
H5
AM933080.1
128 → 127
H8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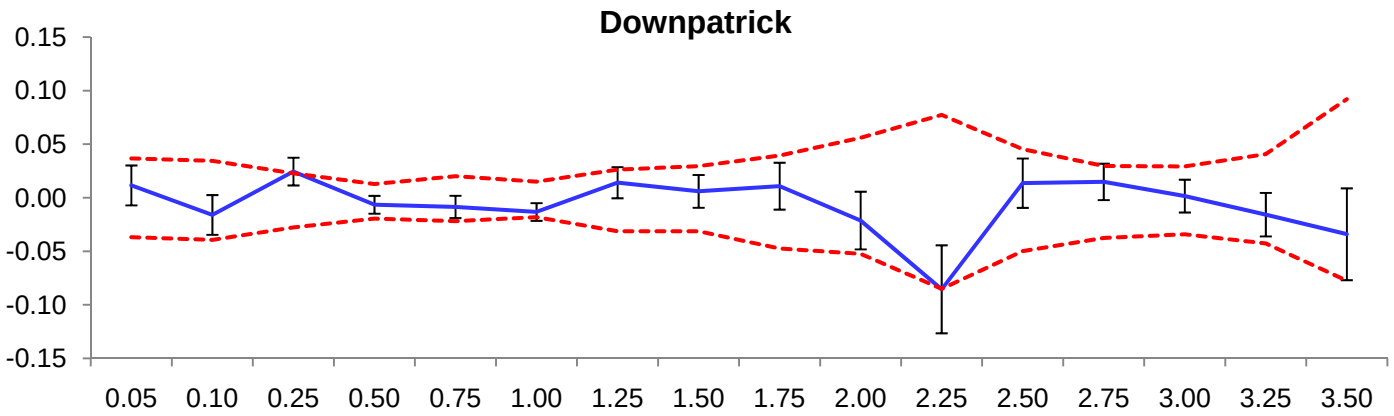
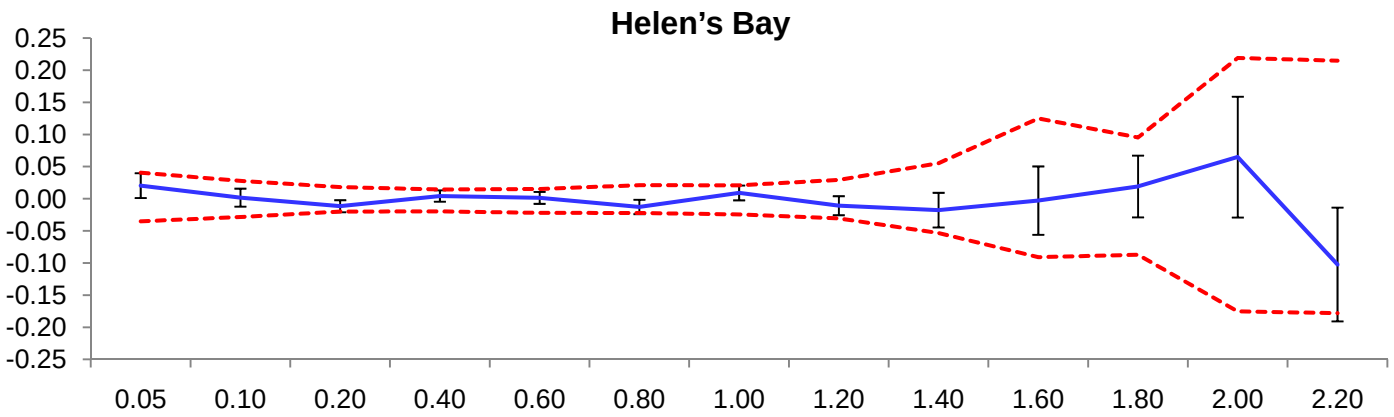
0.10
0.05
0.00
-0.05
-0.10
0.05 0.10 0.50 1.00 1.50 2.00 2.50 3.00 3.50 4.00 4.50 5.00 5.50 6.00 6.50


Downpatrick
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
0.05 0.10 0.25 0.50 0.75 1.00 1.25 1.50 1.75 2.00 2.25 2.50 2.75 3.00 3.25 3.50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0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20
-0.25
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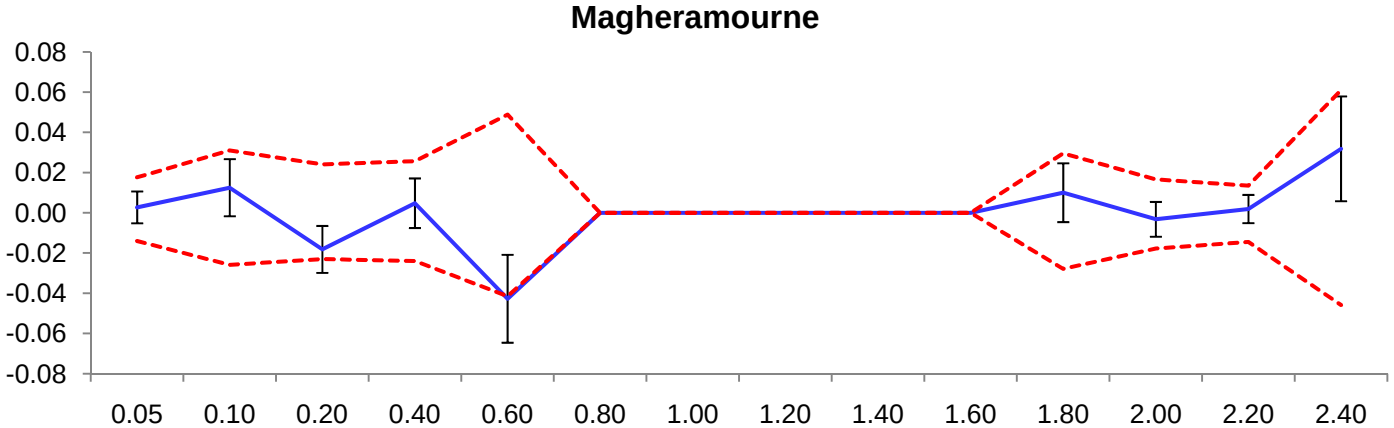
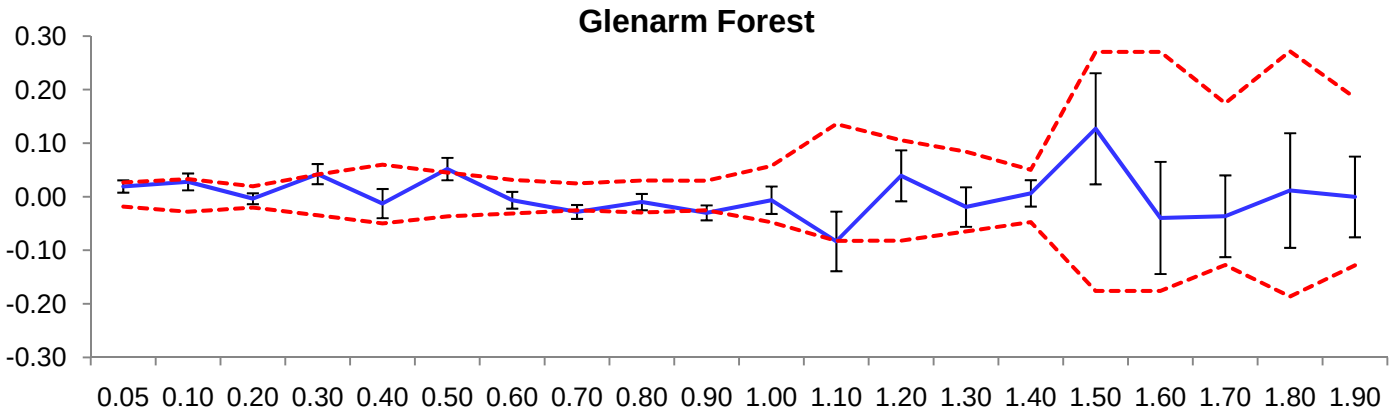
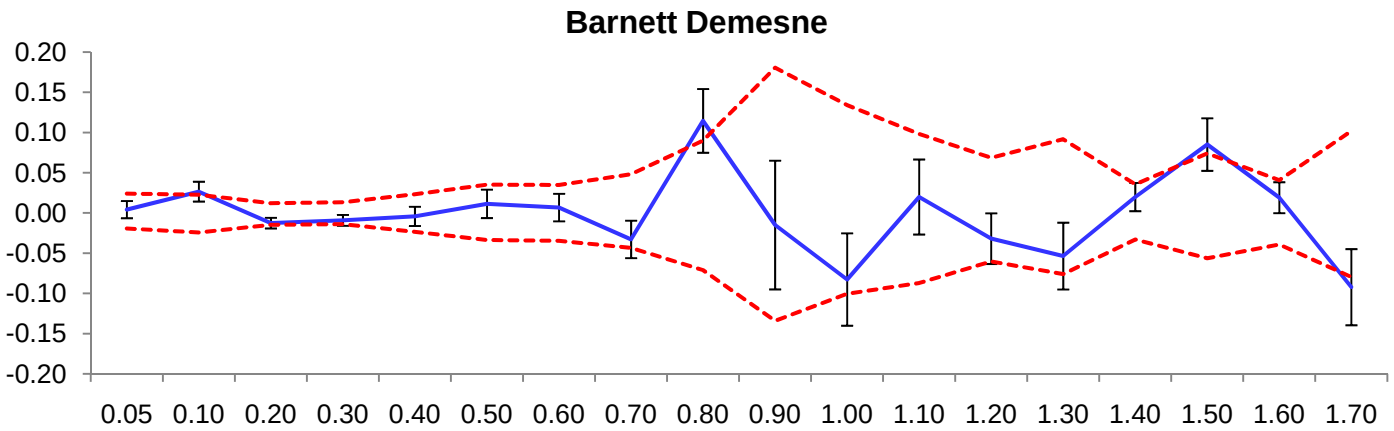

Magheramourne
0.08
0.06
0.04
0.02
0.00
-0.02
-0.04
-0.06
-0.08
0.05 0.10 0.20 0.40 0.60 0.80 1.00 1.20 1.40 1.60 1.80 2.00 2.20 2.40


Glenarm Forest
0.30
0.20
0.10
0.00
-0.10
-0.20
-0.30
0.05 0.10 0.20 0.30 0.40 0.50 0.60 0.70 0.80 0.90 1.00 1.10 1.20 1.30 1.40 1.50 1.60 1.70 1.80 1.90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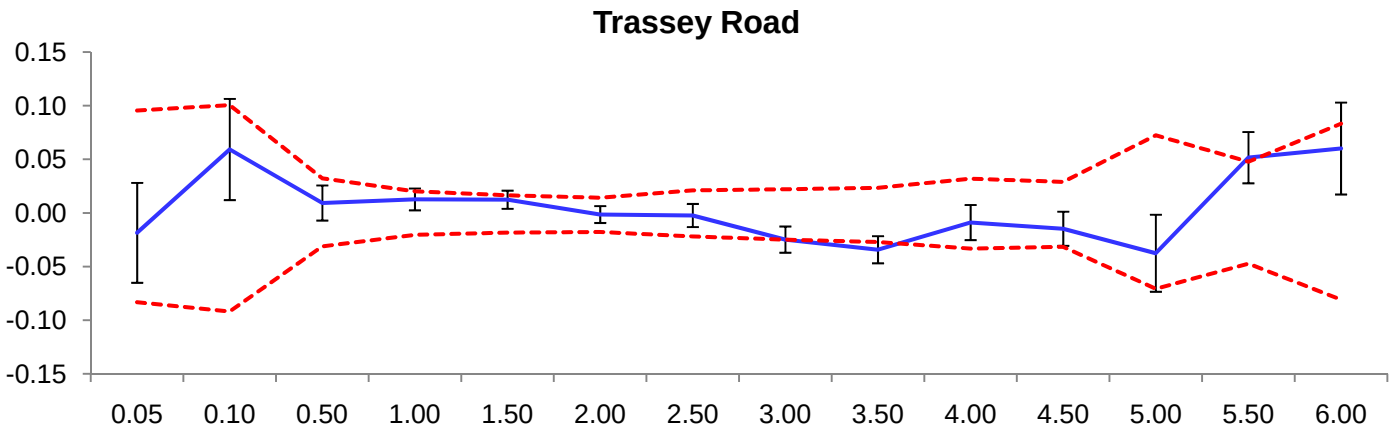
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20
0.05 0.10 0.20 0.30 0.40 0.50 0.60 0.70 0.80 0.90 1.00 1.10 1.20 1.30 1.40 1.50 1.60 1.70


Trassey Road
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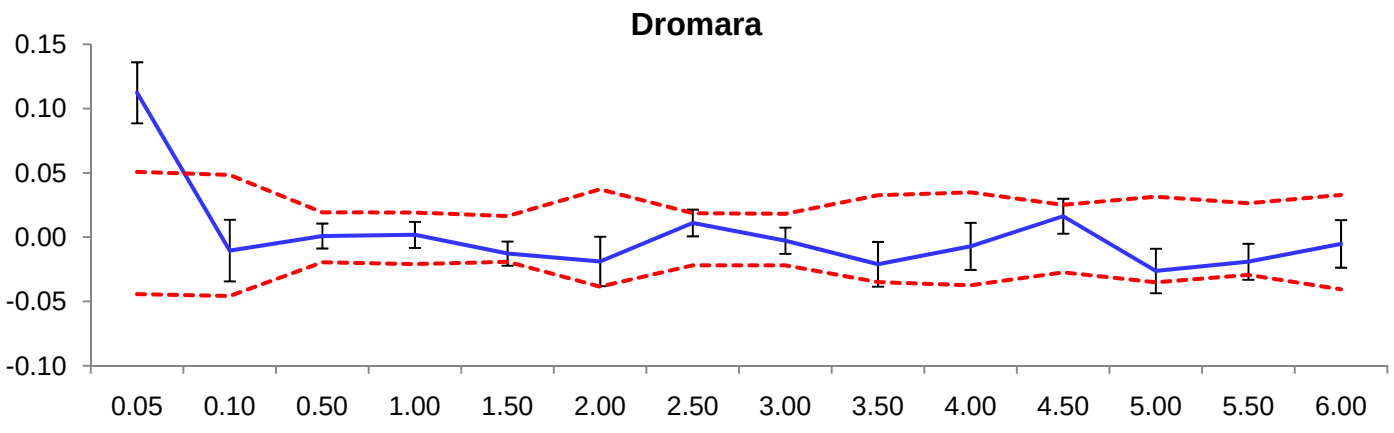
0.10
0.05
0.00
-0.05
-0.10
-0.15
0.05 0.10 0.50 1.00 1.50 2.00 2.50 3.00 3.50 4.00 4.50 5.00 5.50 6.00


Dromara
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0.10
0.05
0.00
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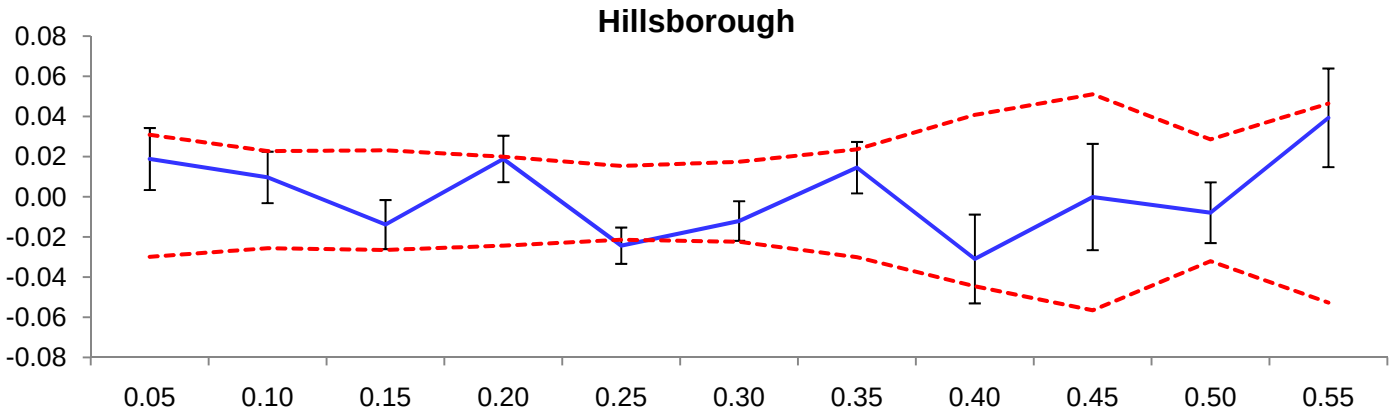
0.05 0.10 0.50 1.00 1.50 2.00 2.50 3.00 3.50 4.00 4.50 5.00 5.50 6.00


Hillsborough
0.08
0.06
0.04
0.02
0.00
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-0.04
-0.06
-0.08
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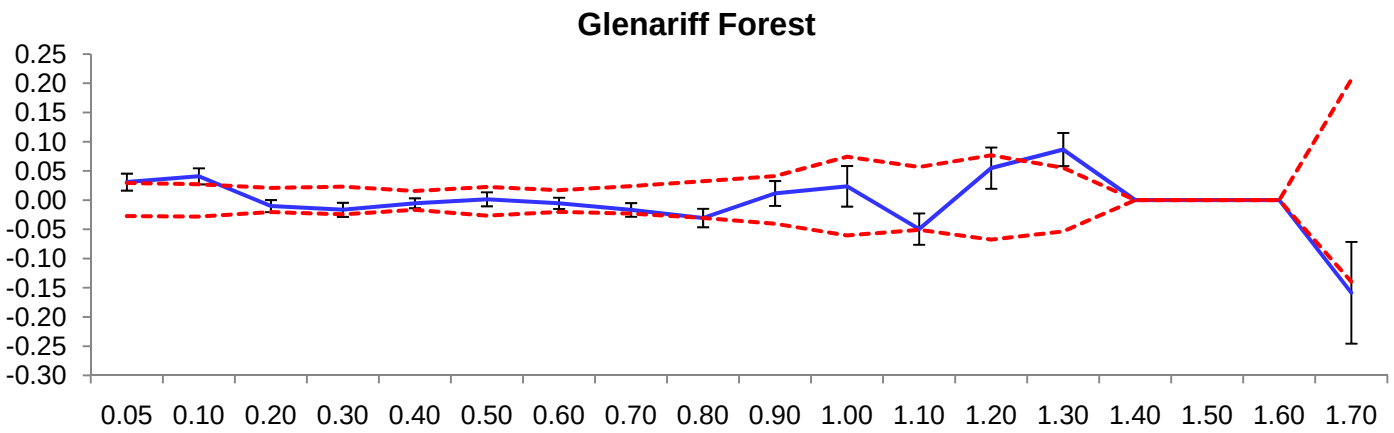
Glenariff Forest
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0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20
-0.25
-0.30
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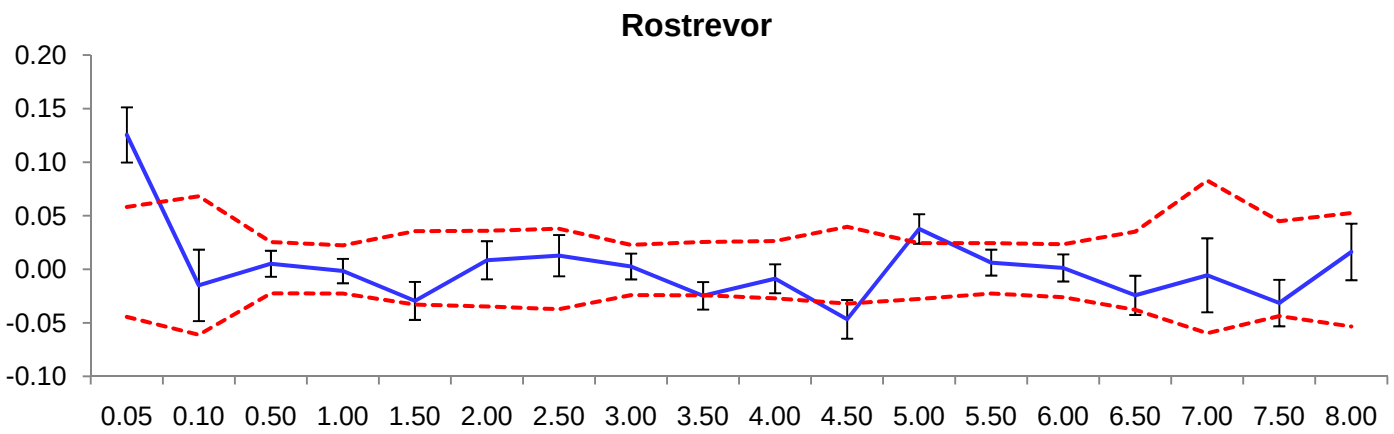
Rostrevor
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0.15
0.10
0.05
0.00
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-0.10
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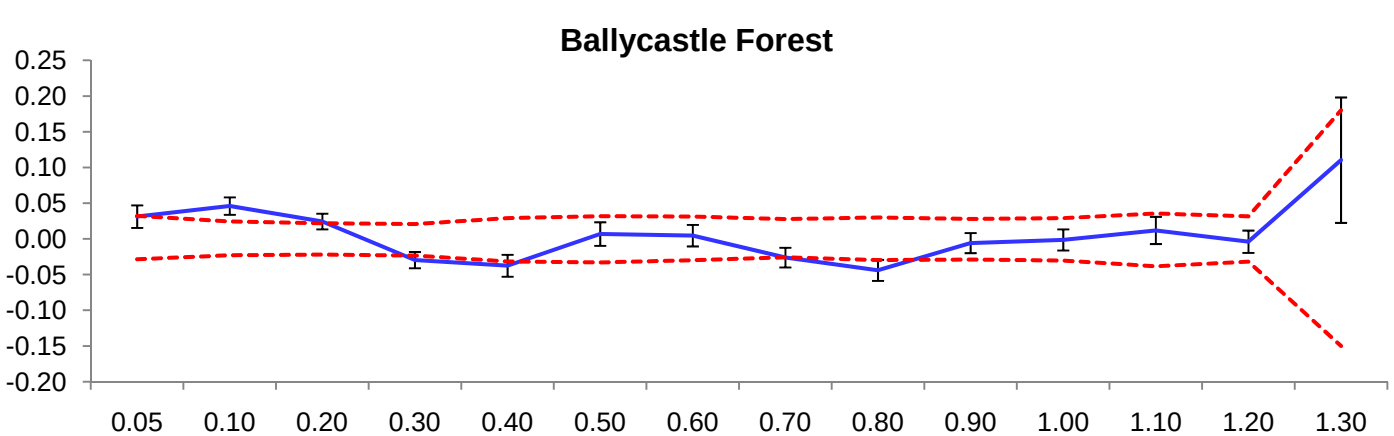
Ballycastle Forest
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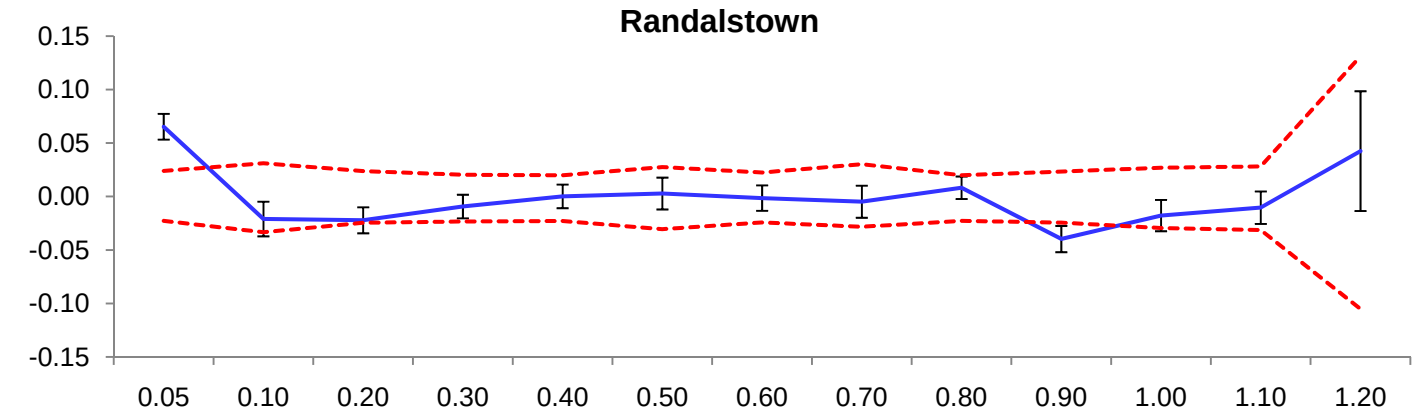
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20
0.05 0.10 0.20 0.30 0.40 0.50 0.60 0.70 0.80 0.90 1.00 1.10 1.20 1.30


Randalstown
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0.05
0.00
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-0.15
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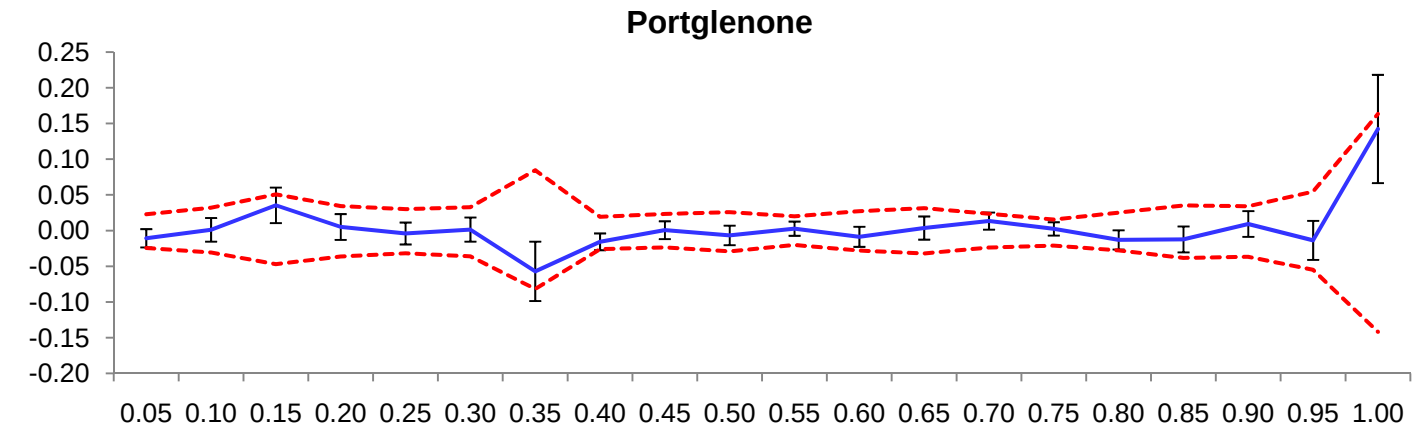
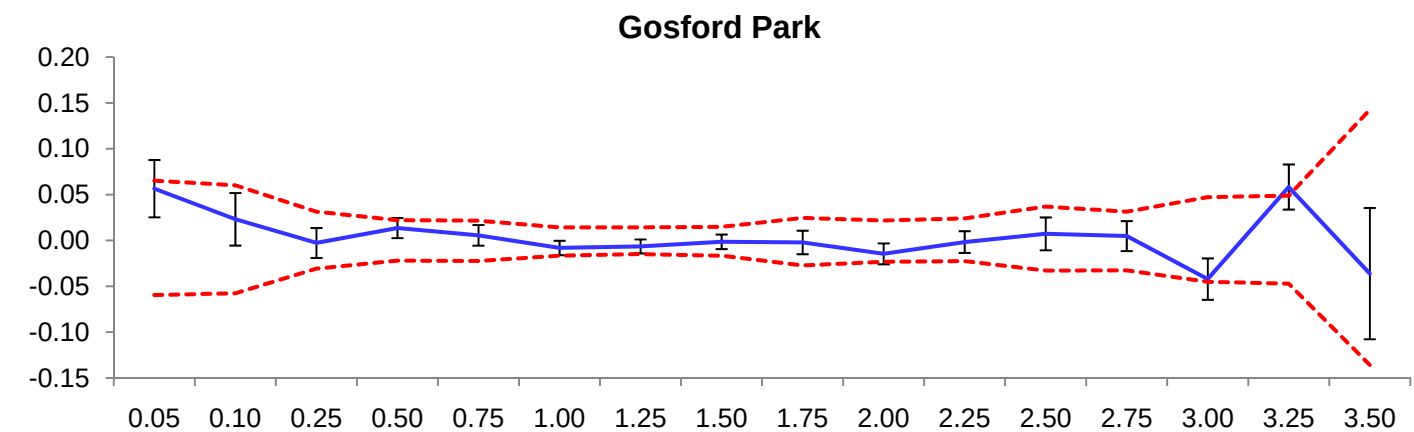

Portglenone
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20
0.05 0.10 0.15 0.20 0.25 0.30 0.35 0.40 0.45 0.50 0.55 0.60 0.65 0.70 0.75 0.80 0.85 0.90 0.95 1.00


Gosford Park
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0.10
0.05
0.00
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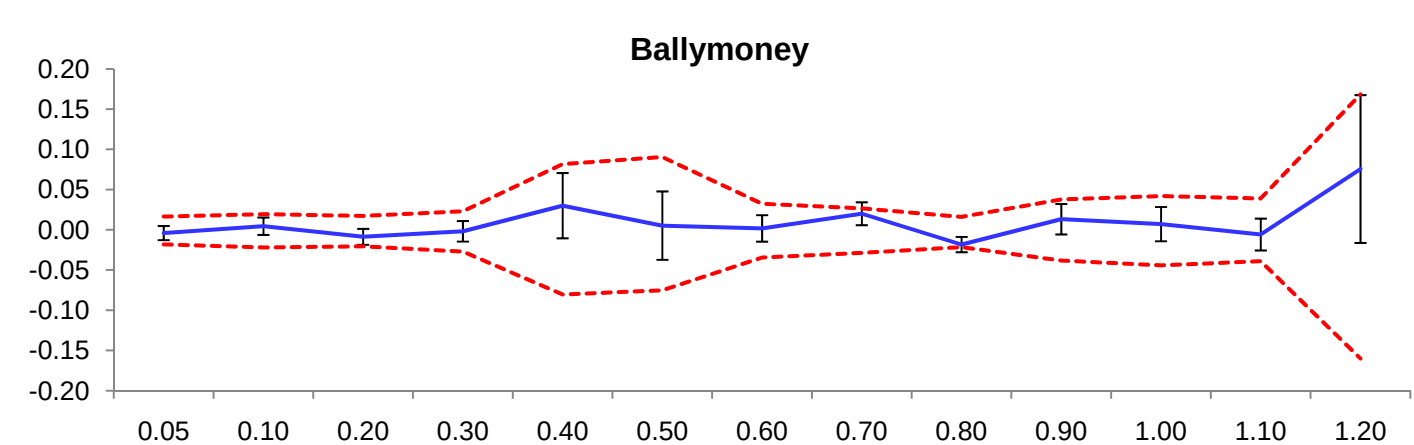
Ballymoney
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0.10
0.05
0.00
-0.05
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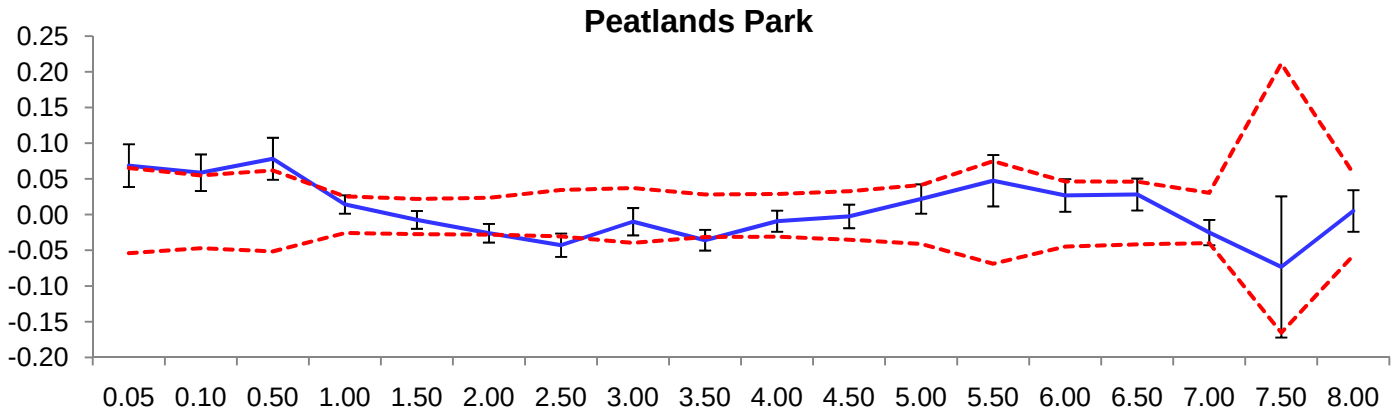
Peatlands Park
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0.15
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0.05
0.00
-0.05
-0.10
-0.15
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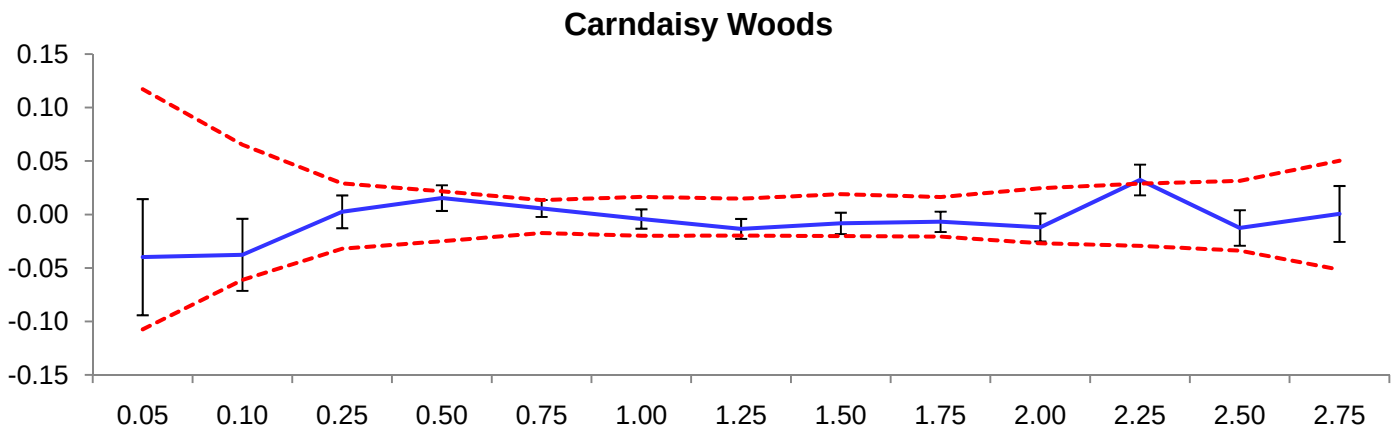
Carndaisy Woods
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0.10
0.05
0.00
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-0.15
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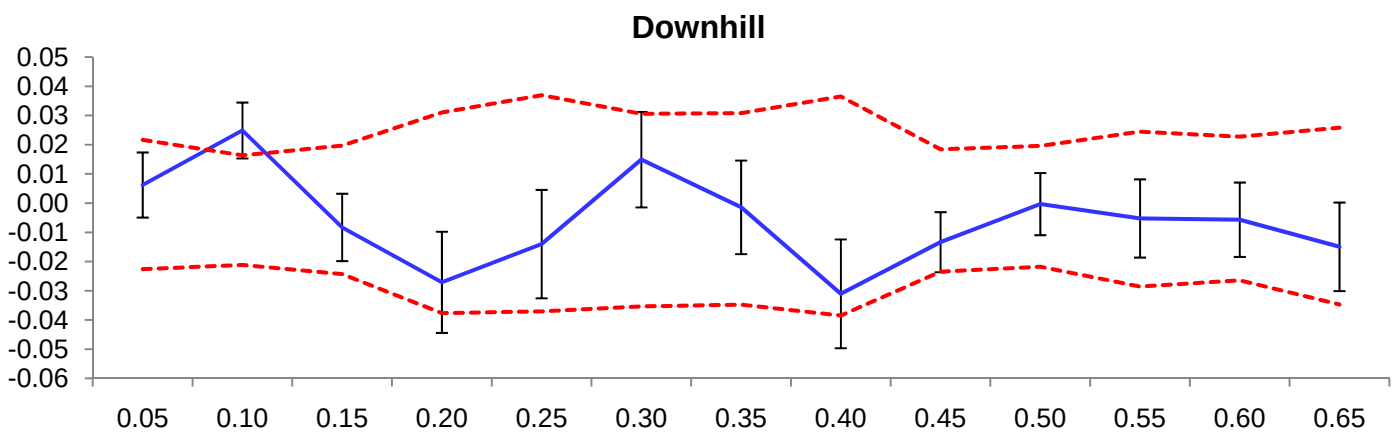
Downhill
0.05
0.04
0.03
0.02
0.01
0.00
-0.01
-0.02
-0.03
-0.04
-0.05
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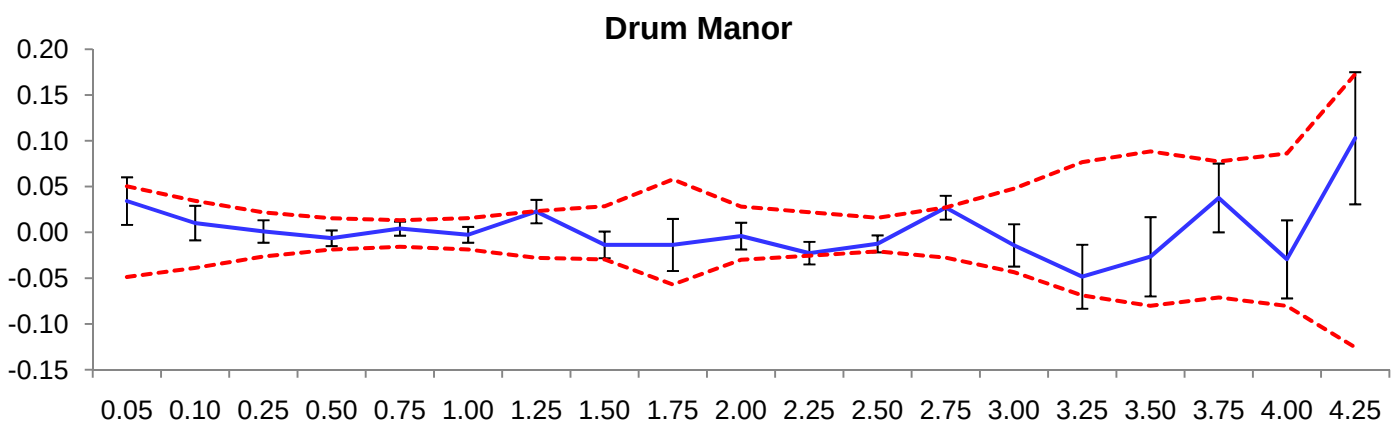
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0.05
0.00
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-0.10
-0.15
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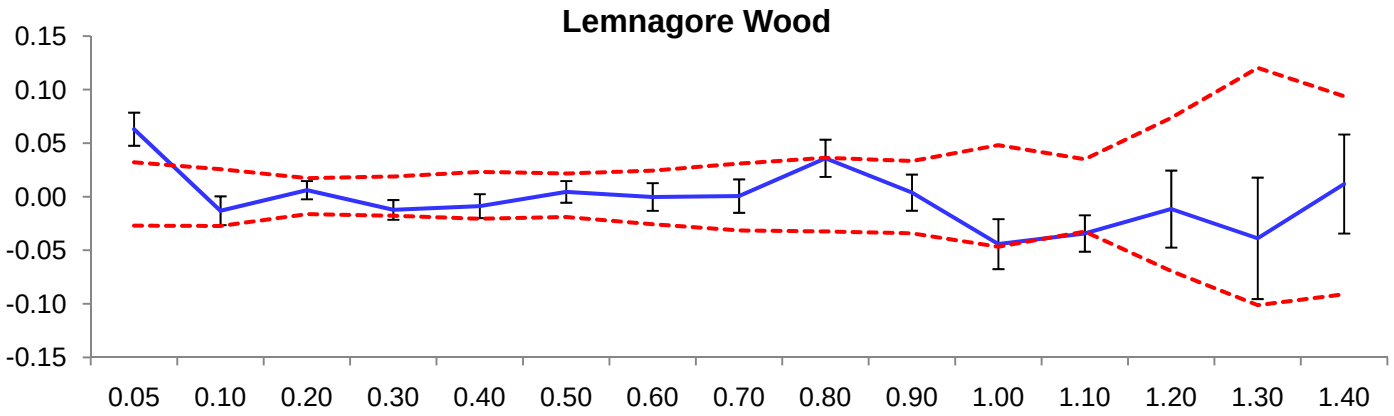
Lemnagore Wood
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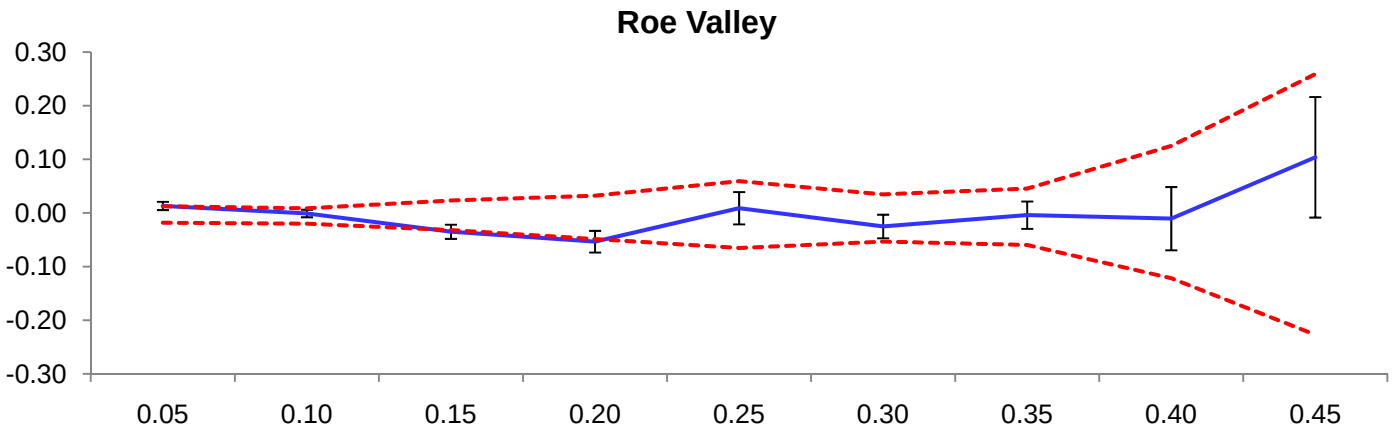
0.10
0.05
0.00
-0.05
-0.10
-0.15
0.05 0.10 0.20 0.30 0.40 0.50 0.60 0.70 0.80 0.90 1.00 1.10 1.20 1.30 1.40


Roe Valley
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0.10
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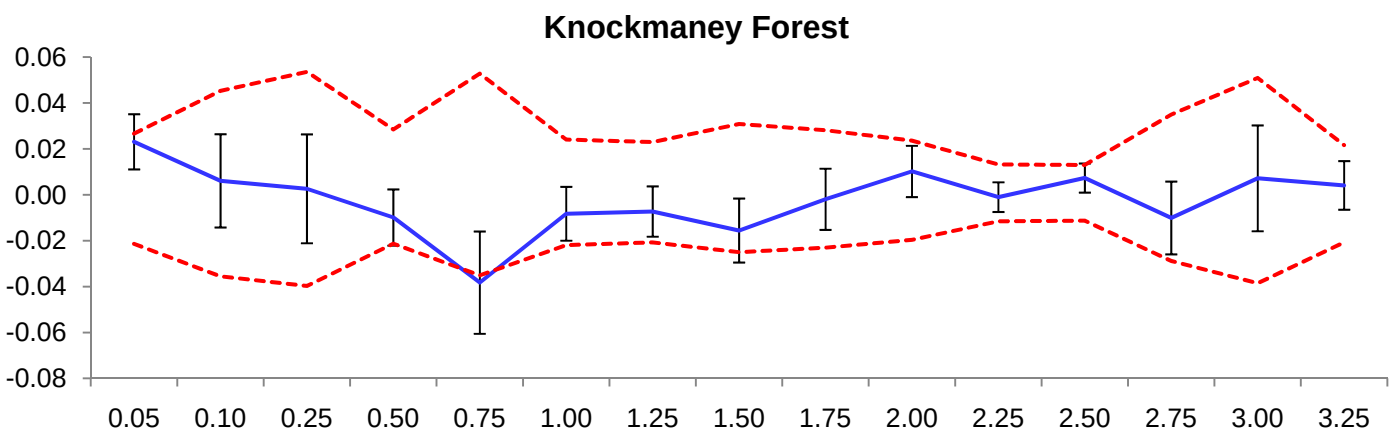
-0.30
0.05 0.10 0.15 0.20 0.25 0.30 0.35 0.40 0.45


Knockmaney Forest
0.06
0.04
0.02
0.00
-0.02
-0.04
-0.06
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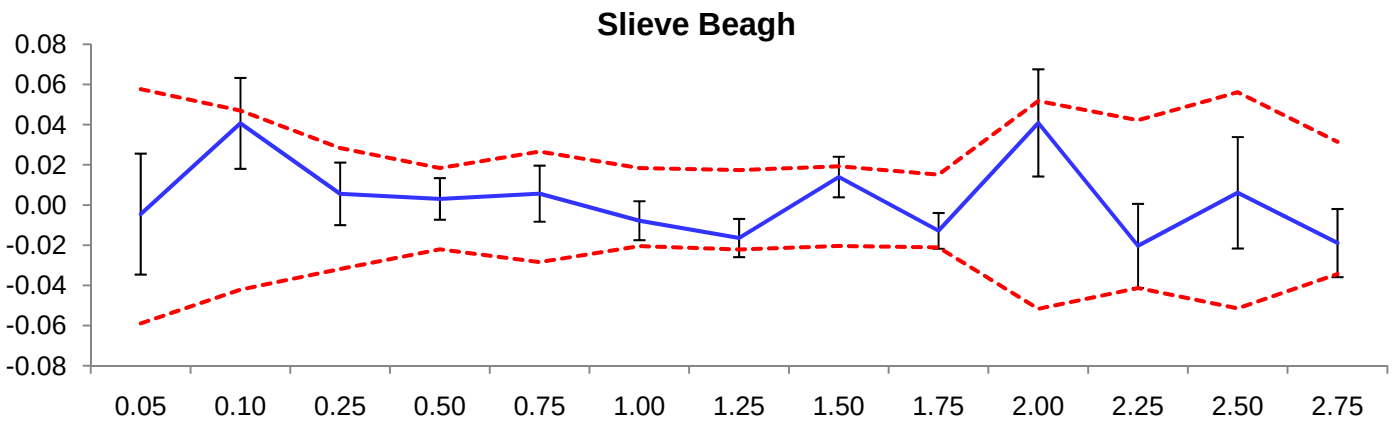
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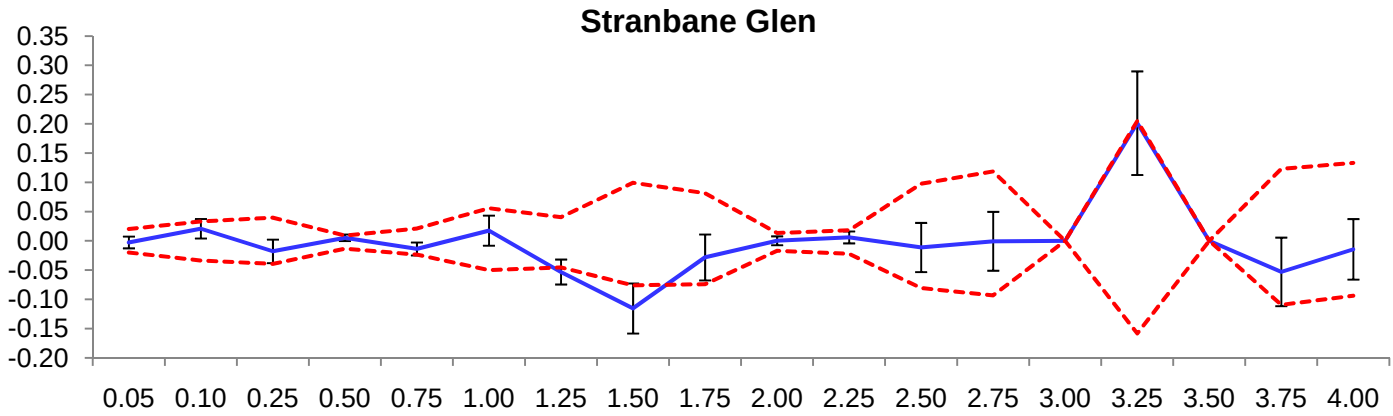
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0.10
0.05
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-0.15
-0.20
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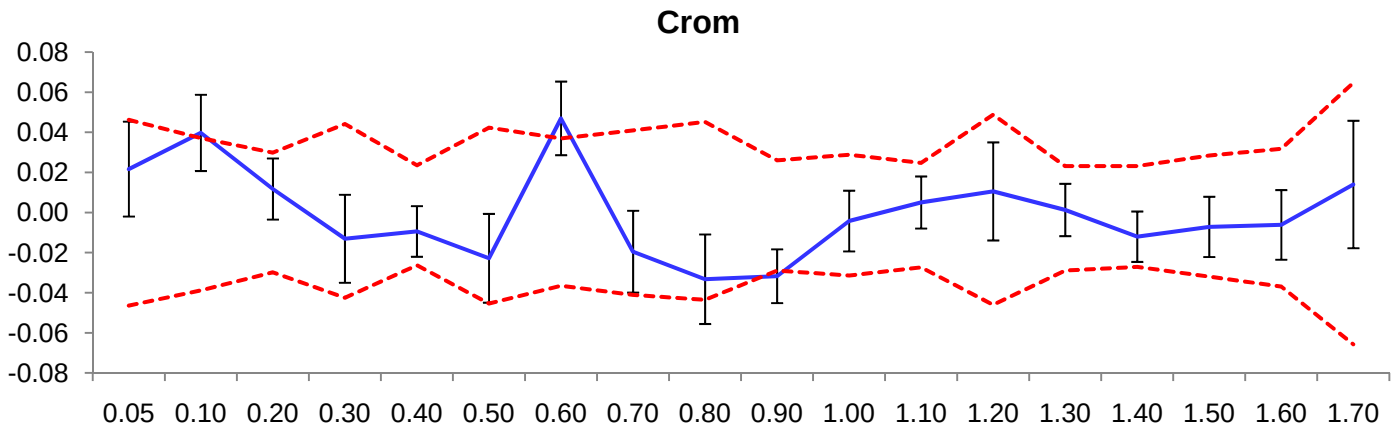
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0.06
0.04
0.02
0.00
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-0.04
-0.06
-0.08
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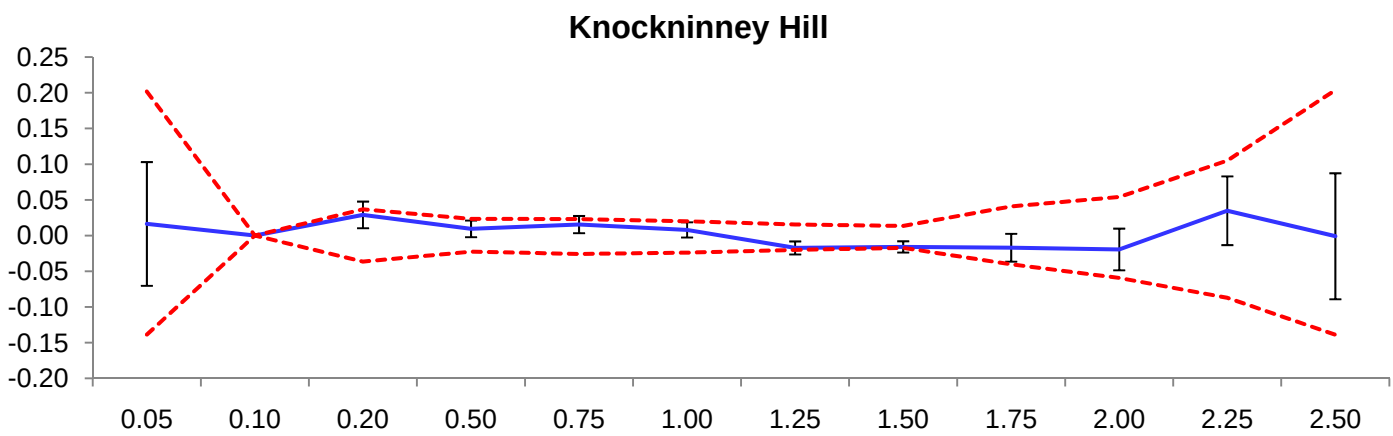
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0.05
0.00
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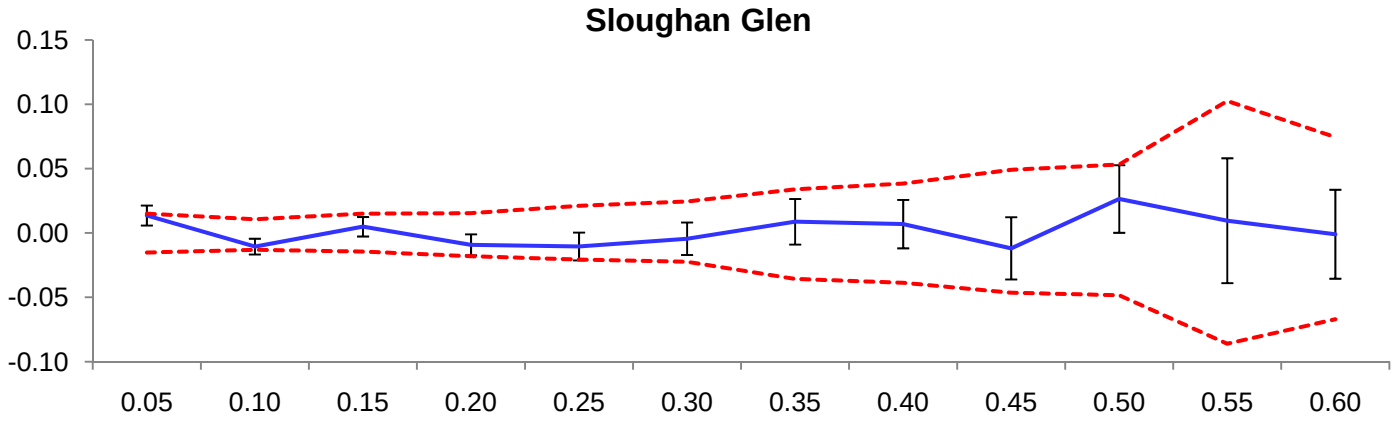
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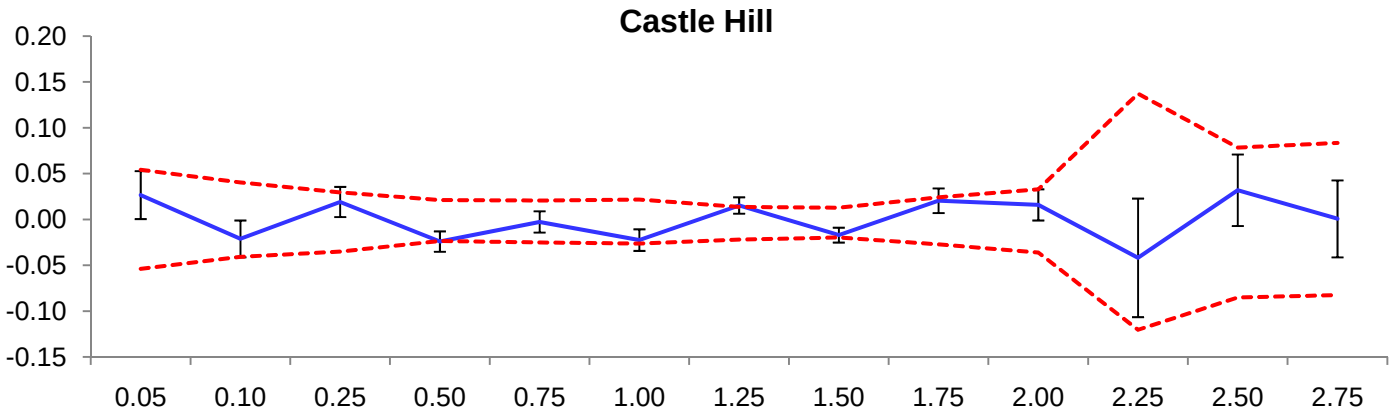
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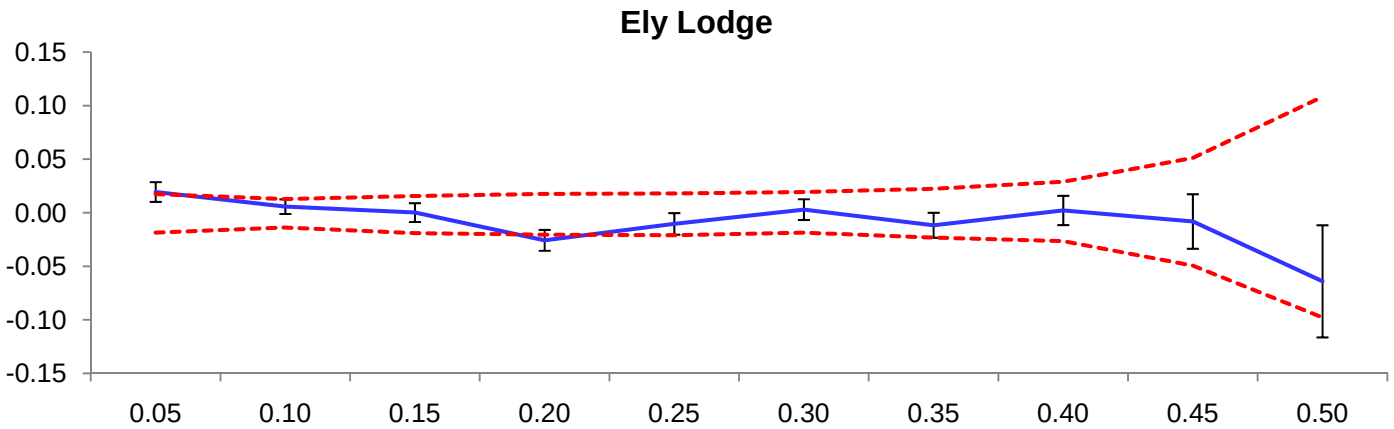
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0.05
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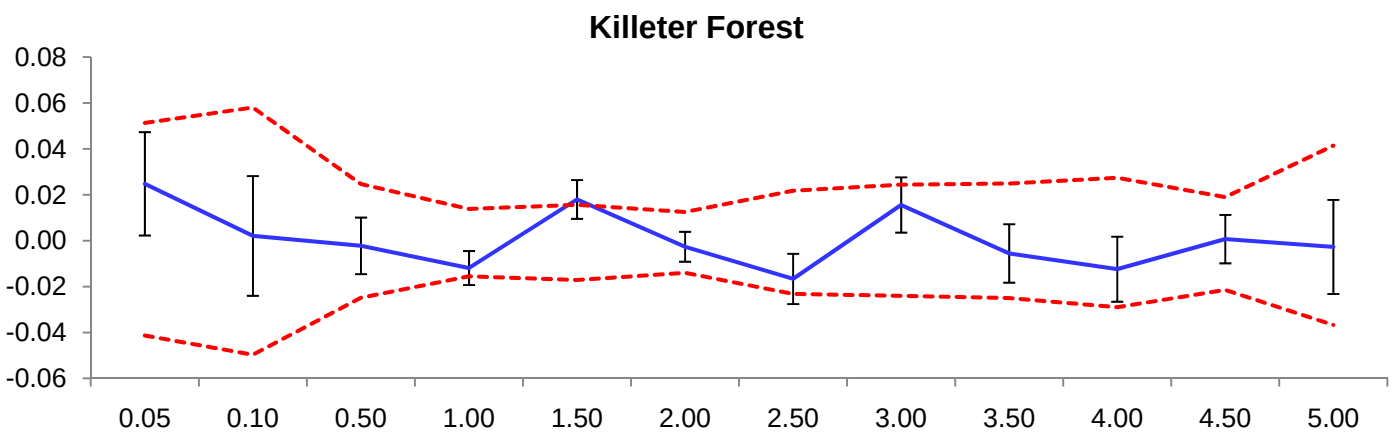
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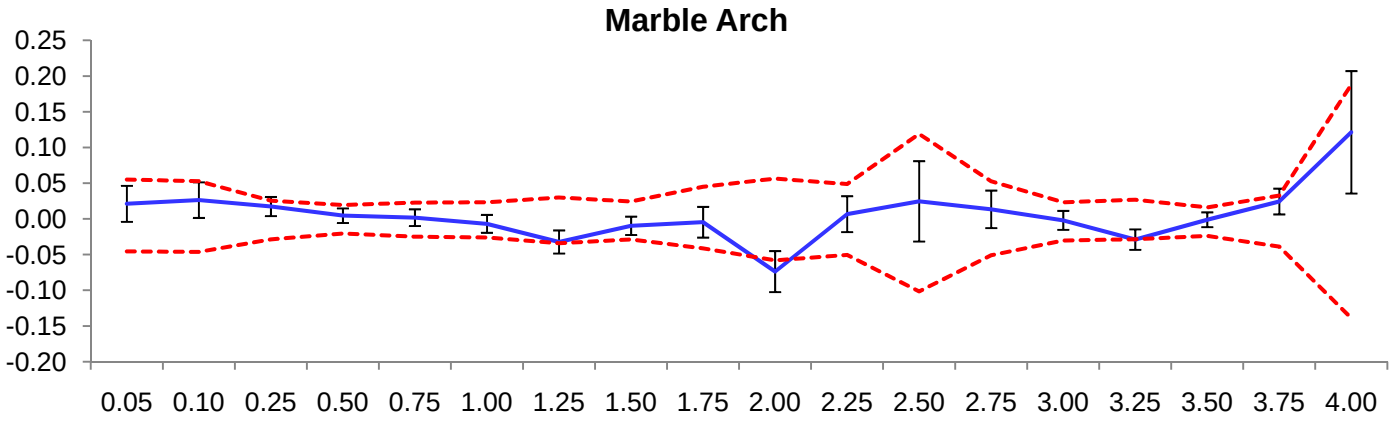
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-0.05
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0.05
0.10
0.25
0.50
0.75
1.00
1.25
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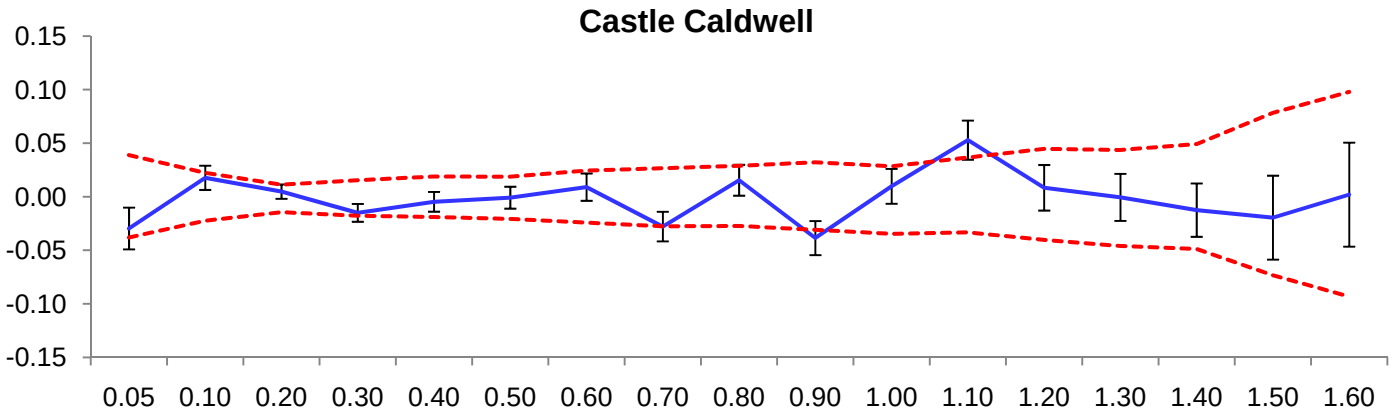
4.00


Castle Caldwell
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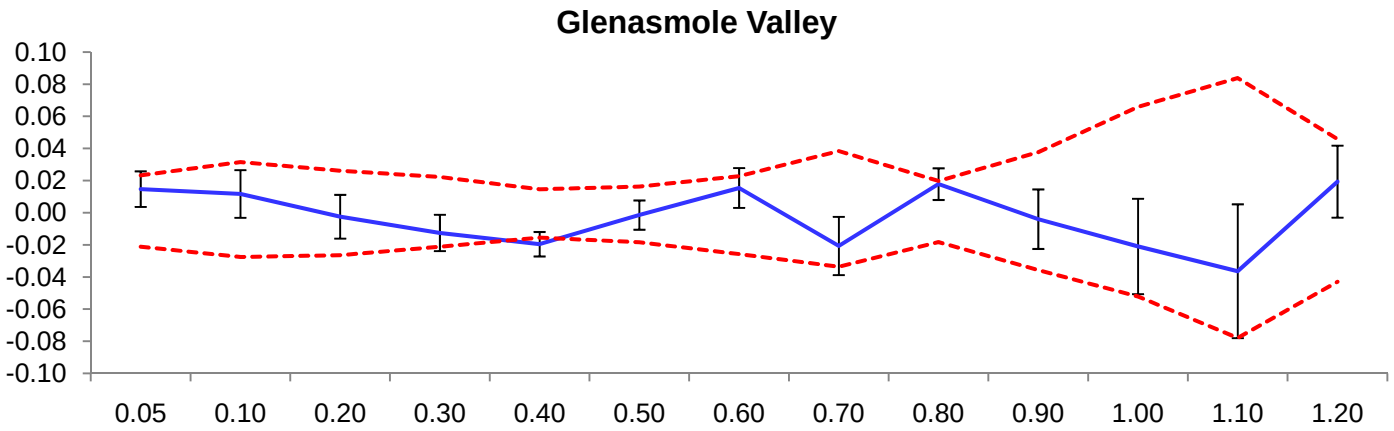
Glenasmole Valley
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
-0.04
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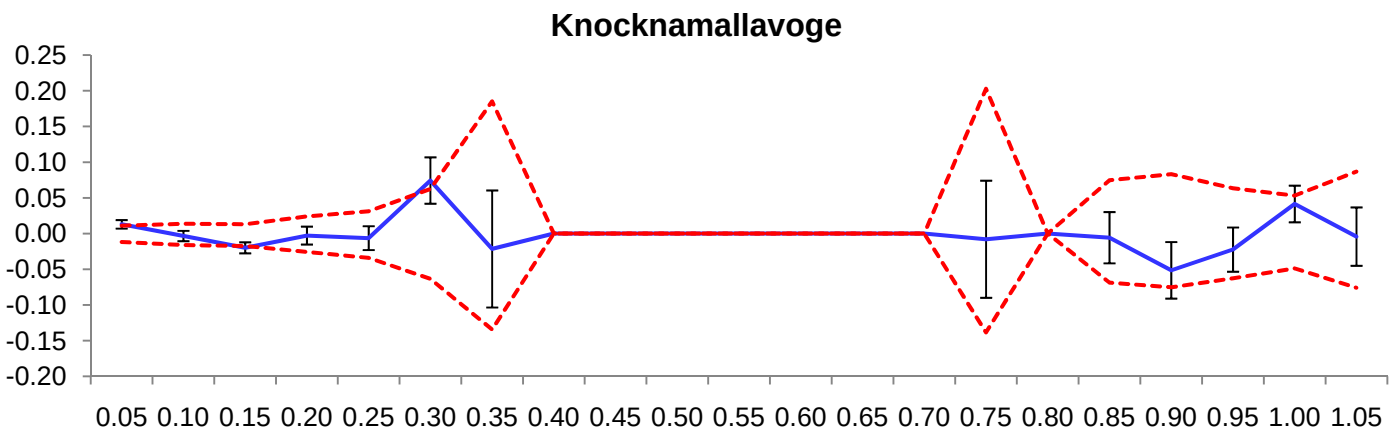
-0.10
0.05 0.10 0.20 0.30 0.40 0.50 0.60 0.70 0.80 0.90 1.00 1.10 1.20


Knocknamallavoge
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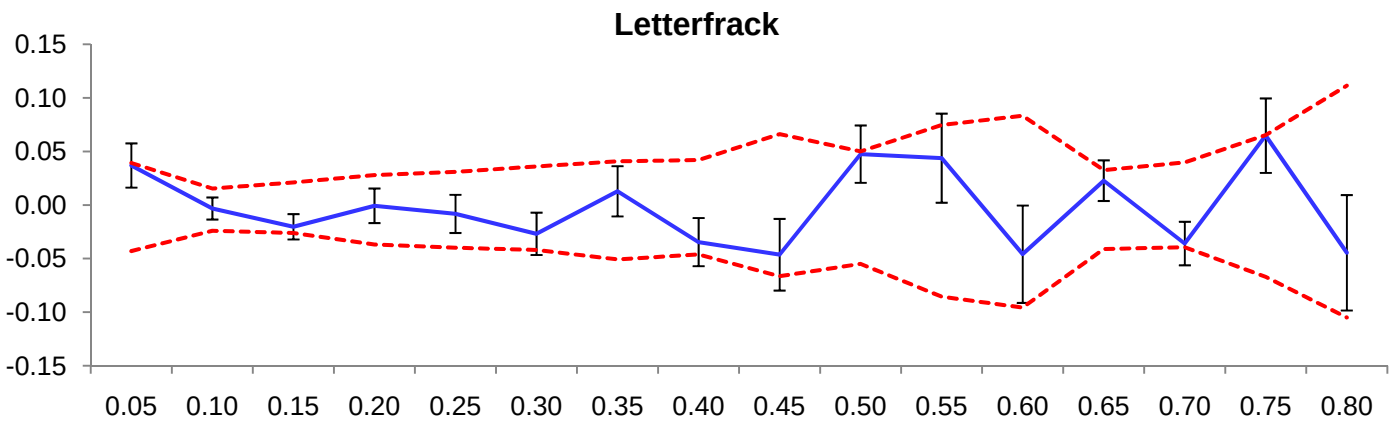
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
-0.20
0.05 0.10 0.15 0.20 0.25 0.30 0.35 0.40 0.45 0.50 0.55 0.60 0.65 0.70 0.75 0.80 0.85 0.90 0.95 1.00 1.05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0.10
0.05
0.00
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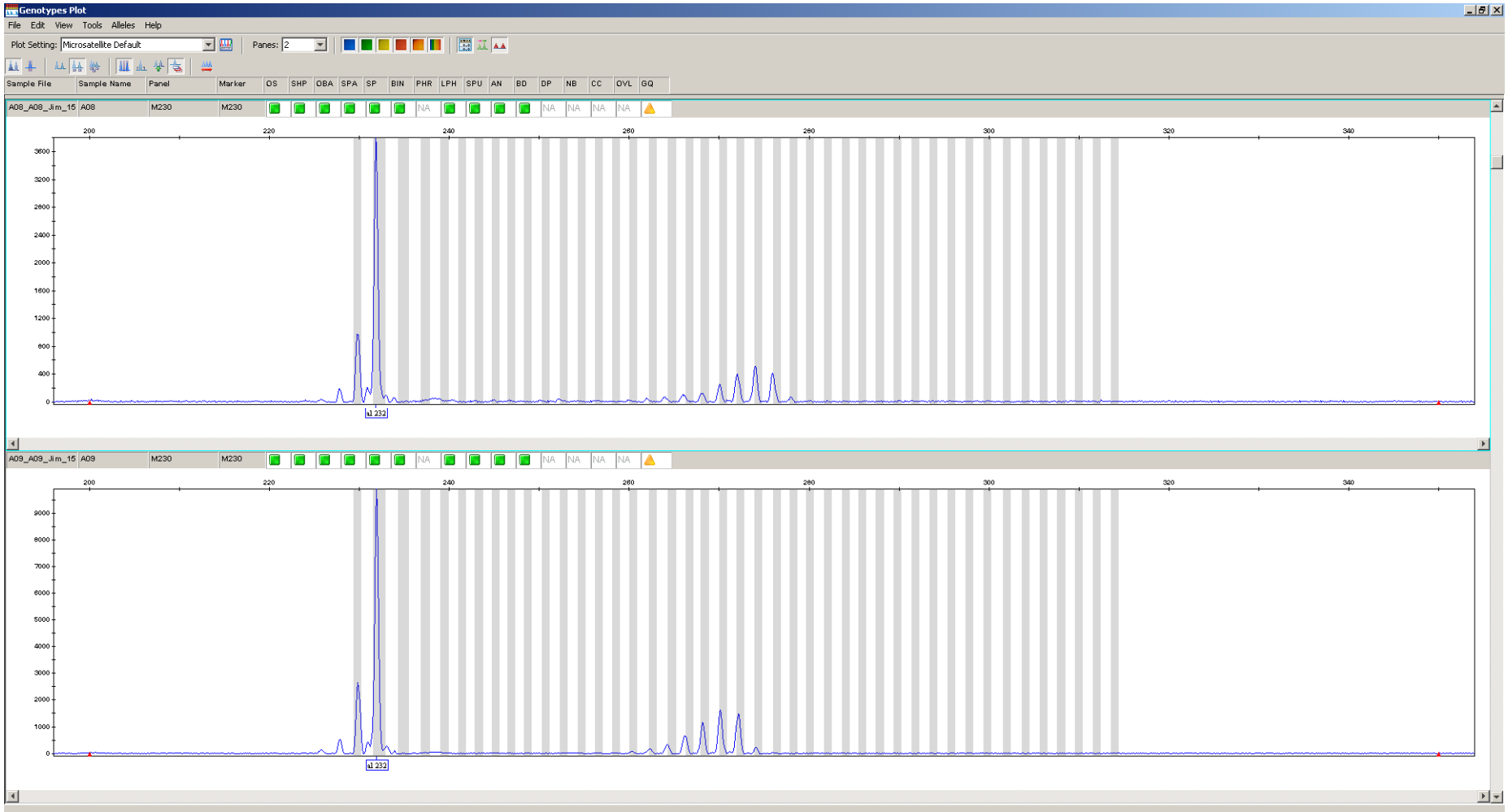
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