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ORIGINAL PAPER

Genetic provenance and best practice woodland management: a case study in native alder (*Alnus glutinosa*)

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Abstract

In recent years, the native woodlands of Europe, including those of Britain and Ireland, have increasingly come under threat from a range of biotic and abiotic factors, and are therefore a conservation priority demanding careful management in order to realise their inherent ecological and cultural benefits. Because the distribution of genetic variation across populations and regions is increasingly considered an important component of woodland management, we carried out a population genetic analysis on black alder (*Alnus glutinosa*) across Northern Ireland in order to inform "best practice" strategies. Our findings suggest that populations harbour high levels of genetic diversity, with very little differentiation between populations. Significant F_{IS} values were observed in over half of the populations analyzed, however, which could reflect inbreeding as a result of the patchy occurrence of alder in Northern Ireland, with scattered, favourable damp habitats being largely isolated from each other by extensive tracts of farmland. Although there is no genetic evidence to support the broad-scale implementation of tree seed zones along the lines of those proposed for native woodlands in Great Britain, we suggest that the localised occurrence of rare chloroplast haplotypes should be taken into account on a case-by-case basis. This, coupled with the identification of populations containing high genetic diversity and that are broadly representative of the region as a whole, will provide a sound genetic basis for woodland management, both in alder and more generally for species that exhibit low levels of genetic differentiation.

ADDITIONAL KEYWORDS: Gene flow, genetic diversity, inbreeding, microsatellites, population genetics

25 **Introduction**

26

27 In recent years, the native woodlands of Europe, including those of Britain and Ireland, have
28 increasingly come under threat from a range of biotic and abiotic factors, including habitat
29 loss and fragmentation, often as a result of land-use change, invasive species, emergent pests
30 and diseases, and global climate change (Rackham 2008). Less than 1.5% of the land area in
31 Britain is occupied by native forest (Brown 1997). Comparable data for Ireland suggest that
32 less than 1% of land area is native woodland and this is continuing to decrease as a result of
33 intensive agriculture and forestry practice (Cross 1998). Our remaining native woodland
34 therefore, is a conservation priority demanding careful management in order to realise its
35 inherent ecological and cultural benefits (Thomas et al. 1997).
36 Black alder (*Alnus glutinosa* [L.] Gaertn.) is a key component of European broadleaved
37 woodlands, which is also found in highly fragmented populations in the extreme northern
38 reaches of northwest Africa (Claessens et al. 2010). The species thrives in damp and riparian
39 habitats, which often results in a patchy distribution, but where it does form stands, it
40 represents an important component of riverine systems by ameliorating erosion (Claessens
41 2003), as well as being one of the few tree species that fixes atmospheric nitrogen via
42 symbiosis with bacteria of the genus *Frankia* (Bond et al. 1954). Its flowers are wind-
43 pollinated catkins which are self-incompatible, and seeds are generally dispersed by wind or
44 water, having cork appendages that can aid floatation for up to a year (McVean 1953).
45 The increased threats to native tree species, including alder, which has been impacted in
46 the last few decades by a disease caused by the oomycete *Phytophthora alni* (Brasier et al.
47 1995), demands the development of "best practice" management strategies. Where
48 restocking or replanting is required, it has been recommended that seed from the same area
49 are used to reflect local provenance (Herbert et al. 1999). The Forestry Commission in Great

50 Britain has consequently delineated “seed zones” to assist in the selection of appropriate
51 material. Such areas, however, are defined primarily by climatic and broad ecological
52 factors, and do not take into account the distribution of genetic variation across populations
53 and regions, which is increasingly considered an important component of woodland
54 management (Müller-Starck et al. 1992; Ennos et al. 1998). A recent study on European ash
55 (*Fraxinus excelsior*) demonstrated that populations across Northern Ireland are represented
56 by a single gene pool, and thus suggests that material for replanting need not be locally
57 sourced (Beatty et al. 2015). In the present study we analyzed populations of alder from
58 across the same region, since increasing deforestation and land use change for agriculture is
59 putting many suitable habitats at risk necessitating the development of rational management
60 programmes.

61 **Materials and methods**

62

63 *Sampling and DNA extraction*

64 Samples were collected from 24 sites across Northern Ireland and one site each in the
65 Republic of Ireland and Scotland that had been previously designated as ancient or semi-
66 natural woodland based on data collected for the Woodland Trust Inventory of ancient and
67 long-established woodland in Northern Ireland (www.backonthemap.org.uk), the National
68 Survey of Native Woodlands 2003-08 in the Republic of Ireland (www.npws.ie) and the
69 Scottish Ancient Woodland Inventory (www.snh.gov.uk; Fig. 1 and Table 1). A single leaf
70 was collected from each of 30 trees per site and stored in silica gel, and GPS coordinates
71 recorded for every tree sampled. DNA was extracted using the CTAB method of Doyle and
72 Doyle (1987). Nuclear genotypes were obtained for between 14 and 29 individuals per
73 population (Table 1; total = 632; mean = 24.308), and chloroplast haplotypes were obtained
74 for between 12 and 30 individuals per population (Table 1; total = 673; mean = 25.885).

75

76 *Genotyping*

77 All trees were genotyped for eleven nuclear and six chloroplast microsatellite loci. For
78 nuclear microsatellite genotyping, we used eleven of the twelve previously reported loci
79 developed for alder, with the exception of locus Ag23, which could not be consistently
80 amplified (Lepais and Bacles 2011). Forward primers included a 19 bp M13 tail
81 (CACGACGTTGTAAAACGAC) and reverse primers included a 7 bp tail (GTGTCTT). The
82 eleven nuclear loci were amplified in three separate multiplex reactions (Ag05, Ag10, Ag14,
83 Ag30 with 6-FAM; Ag01, Ag13, Ag27, Ag35 with HEX; Ag09, Ag20, Ag25 with PET) and
84 combined for capillary electrophoresis.

85 *A. glutinosa* chloroplast sequences in the GenBank database were searched for
86 mononucleotide repeats of nine or more (Provan et al. 2001). Primers were designed using
87 the Primer3 program to amplify the six loci in two multiplexes (Table S1, Supporting
88 Information).
89 PCR was carried out in a total volume of 10 µl containing 100 ng genomic DNA, 5 pmol
90 of 6-FAM-, HEX- or PET-labelled M13 primer, 0.05 pmol of each M13-tailed forward
91 primer, 5 pmol each reverse primer, 1x PCR reaction buffer, 200 µM each dNTP, 2.5 mM
92 $MgCl_2$ and 0.25 U GoTaq Flexi DNA polymerase (Promega, Sunnyvale, CA, USA). PCR
93 was carried out on a MWG Primus thermal cycler (Ebersberg, Germany) using the following
94 conditions: initial denaturation at 94 ^oC for 3 min followed by 40 cycles (30 for chloroplast
95 loci) of denaturation at 94 ^oC for 30 s, annealing at 58 ^oC for 30 s, extension at 72 ^oC for 30 s
96 and a final extension at 72 ^oC for 5 min. Genotyping was carried out on an AB3730xl
97 capillary genotyping system. (Applied Biosystems, Foster City, CA, USA). Allele sizes were
98 scored using the GeneMapper software package (v4.1; Applied Biosystems) using LIZ-500
99 size standards, and were checked by comparison with previously sized control samples.
100 Chromatograms were all inspected visually.
101
102 *Data analysis*
103 GenePop (V3.4; Raymond and Rousset, 1995) was used to test for linkage disequilibrium
104 between nuclear microsatellite loci. To estimate genetic diversity within the populations,
105 levels of observed (H_O) and expected (H_E) heterozygosity, levels of allelic richness (A_R) and
106 fixation indices (F_{IS}) were calculated using the Fstat software package (V2.9.3.2; Goudet,
107 2001). Significance of F_{IS} was determined by 10,000 randomisation steps. Chloroplast
108 microsatellite allele sizes were combined into haplotypes, and levels of genetic diversity (H)
109 based on haplotype frequencies were calculated using the Arlequin software package

110 (V3.5.1.2; Excoffier and Lischer, 2010). To account for differences in sample sizes, levels of
111 haplotype richness (R_h) were also calculated using HAPLOTYPE ANALYSIS (V1.05; Eliades and
112 Eliades 2009).

113 The overall level of genetic differentiation between populations was estimated using Φ_{ST},
114 which gives an analogue of F_{ST} (Weir and Cockerham, 1984) calculated within the analysis
115 of molecular variance (AMOVA) framework (Excoffier et al. 1992) using ARLEQUIN. To
116 further identify possible patterns of genetic structuring, the software package BAPS (V5;
117 Corander et al. 2003) was used to identify clusters of genetically similar populations. The
118 program uses a greedy stochastic optimization algorithm to determine K, the number of
119 clusters. Ten replicates were run for all possible values of K up to $K = 26$, the number of
120 populations sampled. Multiple independent runs always gave the same outcome.

121 **Results**

122

123 No significant evidence of consistent linkage disequilibrium (i.e. involving the same loci)
124 was detected between any of the eleven nuclear microsatellites analysed (35 out of 1430
125 tests). Between five (Ag20) and 19 (Ag14) alleles were detected per locus, with a total of
126 130 (mean = 11.818 per locus; Table 1; Figures S1-S11, Supporting Information). Within
127 populations, levels of allelic richness (A_R) averaged over loci ranged from 3.786 (Gortin
128 Glen) to 5.056 (Rostrevor), with a mean value of 4.664 (Table 1). Thirteen private alleles
129 (10% of the total number of alleles) were detected, with the number per population ranging
130 from zero to two (see Figures S1-S11, Supporting Information). The majority (11) of these
131 were restricted to a single individual, with one of those remaining being found in two
132 individuals and the other found in three individuals from the Roe Valley population (16/632 =
133 2.5% of all trees studied). Levels of observed (H_O) and expected (H_E) heterozygosity ranged
134 from 0.530 (Gortin Glen) to 0.680 (Ballinderry Bridge; mean = 0.612), and from 0.584
135 (Gortin Glen) to 0.708 (Marble Arch; mean = 0.663) respectively. The heterozygote deficit
136 observed in all of the populations gave rise to F_{IS} values which were significantly different
137 from zero in 17 of the 26 populations studied, ranging from 0.008 (Lough Beg) to 0.155
138 (Correl Glen; mean = 0.078).

139 Four of the six chloroplast microsatellite loci studied were polymorphic, exhibiting either
140 two or three alleles (Table S1, Supporting Information). Combining allele sizes across loci
141 gave seven haplotypes. Two of these (H1 and H2) were found in the vast majority (648 out
142 of 673) of the trees studied (Table 1). Of the remainder, H3 was found in 19 individuals, 17
143 of which belonged to the Fardross Forest population. Levels of haplotype diversity (H)
144 ranged from 0.069 (Castle Archdale) to 0.582 (Crom). Levels of haplotype richness (R_h)
145 ranged from 0.414 (Castle Archdale) to 2.196 (Fardross Forest).

146 Levels of population differentiation based on the nuclear and chloroplast markers were

147 Φ_{ST} = 0.0195 and Φ_{ST} = 0.1864 respectively (Table 2). Population-pairwise Φ_{ST} values

148 ranged from -0.034 (Hillborough / Ballinderry Bridge) to 0.078 (Portaferry / Lough Beg) for

149 the nuclear microsatellites, and from -0.058 (Hillsborough / Gortin Glen) to 0.850 (Portaferry

150 / Castle Archdale). The BAPS analysis assigned 25 of the 26 populations to the same genetic

151 cluster, the exception being the Glenarriff population.

152 **Discussion**

153

154 The findings of the present study suggest that alder populations across Northern Ireland
155 harbour high levels of genetic diversity, with very little differentiation between populations.
156 It is somewhat difficult to put the levels of genetic diversity observed into any significant
157 context, since only a single population genetic analysis of alder using microsatellites has been
158 carried out. In it, a study on fragmented populations from Northern Africa (Lepais et al.
159 2013), gene diversity values (equivalent to expected heterozygosity) ranged from 0.48 to 0.59
160 for diploid populations, with a mean value of 0.54 (unusual tetraploid populations from
161 Morocco exhibited higher values). These were lower than the values observed in Northern
162 Ireland in this study, which ranged from 0.584 to 0.699, with a mean of 0.663. This value is
163 similar to that exhibited by the sole northern population analysed in the previous study, which
164 was from Perthshire in Scotland (0.67). We also genotyped 23 individuals from a population
165 in Tarbet, Scotland, which had H_E = 0.662, as well as 24 individuals from Coolure, Co.
166 Westmeath (H_E = 0.617). Thus, the levels of genetic diversity in Northern Irish populations
167 of alder would appear to be comparable to those from the rest of Great Britain and Ireland.
168 Significant F_{IS} values were found in over half of the populations analyzed, and were
169 generally in excess of those reported in both an allozyme study on Slovakian populations
170 (Gömöry and Paule 2002) and a microsatellite study on a single Scottish population (Lepais
171 and Bacles 2011), but lower than that reported in an allozyme study in Poland (Mejnartowicz
172 2008). This could be a result of inbreeding, reflecting the patchy occurrence of alder in
173 Northern Ireland, with scattered, favourable damp habitats being largely isolated from each
174 other by extensive tracts of farmland. Nevertheless, this is not entirely consistent with the
175 apparent high levels of gene flow, most likely via pollen given the low levels of genetic
176 differentiation observed at the nuclear microsatellite loci. Alternative explanations could

177 involve Wahlund effects, as a result of substructuring within populations, and/or unevenness
178 in patterns of recruitment ("sweepstakes recruitment").

179 Analysis of genetic structuring based on nuclear microsatellites did not reveal any obvious
180 patterns. Overall levels of differentiation were low, with around two percent of the total
181 diversity being partitioned between populations (Table 2), a figure similar to that in
182 Slovakian populations (Gömöry and Paule 2002). Consequently, all but one of the
183 populations (including those from the Republic of Ireland and Scotland) were assigned to a
184 single genetic cluster in the BAPS analysis. Assignment of the Glenarriff population to an
185 alternative cluster is most likely an artefact of the BAPS algorithm, which has been shown
186 previously to tend to overestimate the true number of clusters, particularly where levels of
187 differentiation are low (Latch *et al* 2006). An examination of the allele frequencies at each of
188 the eleven loci (Figures S1-S11, Supporting Information) indicates that any difference in the
189 Glenarriff population is largely due to slight differences in the frequency of a low number of
190 alleles at a few of the loci.

191 Chloroplast markers tend to reveal more genetic structuring in natural plant populations
192 due to their lower effective population size and, in angiosperms, being maternally inherited
193 and thus dispersed via seed (Provan *et al.* 2001). This was reflected in Φ_{ST} values for
194 chloroplast microsatellites that were an order of magnitude higher than those for nuclear
195 microsatellites, indicating that 19% of the genetic variation was partitioned between
196 populations. Nevertheless, this genetic variation was not partitioned geographically on any
197 broad scale. Thus, from a management point of view, any recommendations concerning
198 restocking should be taken at the population or local level, particularly where the population
199 in question contains a high proportion of genotypes not found elsewhere. An obvious
200 example of this is the Fardross Forest population, which is dominated by an otherwise
201 relatively rare haplotype. In the event of complete loss of trees from a woodland, for

202 example following a catastrophic disease outbreak, it would be prudent to use some form of
203 "genetic matching" to identify populations with broadly similar haplotype compositions.
204 Additionally, as a general recommendation for good conservation genetic practice, particular
205 attention should be paid to populations of a species that contain high levels of diversity and
206 exhibit multiple haplotypes (Allendorf and Luikart 2007). In alder, for example, the Fardross
207 Forest population contains four of the five non-unique haplotypes found in Northern Ireland,
208 including the two haplotypes that dominate the remaining populations, with the remaining
209 non-unique haplotype being found in a single individual from each of the extreme
210 southeasterly populations (Portaferry and Hollymount). Such smaller-scale geographical
211 localization of haplotypes represents a further factor that should be taken into account when
212 assessing potential material for restocking.

213 The findings of the present study provide a good framework for the development of best
214 practice management for native woodlands, particularly for species that exhibit low levels of
215 genetic differentiation, including many tree species. Although there is no genetic evidence in
216 alder or in ash (Beatty et al. 2015) to suggest the broad-scale implementation of seed zones
217 along the lines of those proposed for Great Britain, there is enough evidence to suggest that
218 the localised occurrence of rare haplotypes should be taken into account on a case-by-case
219 basis. This, coupled with the identification of populations containing high genetic diversity
220 and that are broadly representative of the region as a whole, will provide a sound genetic
221 basis for woodland management.

222 **Acknowledgements**

223

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227 **Data archiving statement**

228

229 All data will be deposited in DRYAD on acceptance.

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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 (* P<0.05; ** P<0.01; *** P<0.001; NS – non-significant); H1-H7 – frequency of chloroplast haplotypes; *H* – gene diversity; R_h – haplotypic richness.

No	*Name*	*Lat*	*Long*	*Nuclear*						*Chloroplast*									
		(N)	*(W)*	N	A_R	P	H_O	H_E	F_{IS}	N	*H1*	*H2*	*H3*	*H4*	*H5*	*H6*	*H7*	H	R_h
1	Portaferry	54.391	5.565	14	3.838	-	0.614	0.628	0.023^{NS}	15	1	13	-	1	-	-	-	0.257	1.600
2	Hollymount	54.322	5.751	28	4.762	1	0.577	0.666	0.136***	29	21	6	-	1	-	1	-	0.446	1.802
3	Glenarm	54.964	5.955	24	4.646	-	0.647	0.674	0.041^{NS}	28	13	15	-	-	-	-	-	0.516	1.000
4	Lagan Valley	54.552	5.960	22	4.765	-	0.627	0.671	0.067*	26	14	12	-	-	-	-	-	0.517	1.000
5	Hillsborough	54.459	6.083	20	4.530	-	0.598	0.633	0.057^{NS}	25	20	5	-	-	-	-	-	0.333	0.976
6	Glenarriff	55.039	6.085	28	4.133	1	0.575	0.619	0.072*	30	17	13	-	-	-	-	-	0.508	1.000
7	Rostrevor	54.093	6.190	26	5.056	1	0.663	0.694	0.047^{NS}	29	25	4	-	-	-	-	-	0.246	0.900
8	Rea's Wood	54.705	6.229	21	4.936	2	0.617	0.699	0.120***	28	8	19	1	-	-	-	-	0.474	1.424
9	Clare Glen	54.354	6.428	27	5.017	-	0.596	0.672	0.115***	29	17	12	-	-	-	-	-	0.502	1.000
10	Lough Beg	54.802	6.485	26	4.372	1	0.621	0.625	0.008^{NS}	29	8	21	-	-	-	-	-	0.414	0.994
11	Ballinderry Bridge	54.669	6.521	29	4.964	-	0.680	0.696	0.023^{NS}	30	16	14	-	-	-	-	-	0.515	1.000
12	Peatlands Park	54.483	6.612	22	4.765	-	0.589	0.667	0.120**	21	13	8	-	-	-	-	-	0.495	1.000
13	Mount Sandel	55.100	6.646	22	4.587	-	0.598	0.668	0.107**	29	14	15	-	-	-	-	-	0.517	1.000
14	Drum Manor	54.639	6.815	24	4.836	1	0.599	0.647	0.076**	28	12	16	-	-	-	-	-	0.508	1.000
15	Roe Valley	55.025	6.939	24	4.522	1	0.576	0.639	0.101**	27	17	10	-	-	-	-	-	0.484	1.000
16	Ness Wood	54.947	7.181	27	4.990	-	0.632	0.686	0.081**	28	15	13	-	-	-	-	-	0.516	1.000
17	Gortin Glen	54.667	7.233	24	3.786	1	0.530	0.584	0.098*	12	9	3	-	-	-	-	-	0.409	1.000
18	Fardross Forest	54.374	7.268	22	4.574	-	0.576	0.665	0.137***	25	5	2	17	-	1	-	-	0.510	2.196
19	Crom	54.170	7.451	25	4.912	-	0.673	0.686	0.020^{NS}	26	14	10	1	-	-	-	1	0.582	1.912
20	Belle Isle	54.245	7.564	25	4.955	-	0.665	0.687	0.032^{NS}	26	13	13	-	-	-	-	-	0.520	1.000

Table 1 (Continued)

No	*Name*	*Lat (N)*	*Long (W)*	Nuclear						Chloroplast									
				N	A_R	P	H_O	H_E	F_{IS}	N	*H1*	*H2*	*H3*	*H4*	*H5*	*H6*	*H7*	H	R_h
21	Sloughan Glen	54.622	7.564	27	4.613	2	0.597	0.655	0.091**	26	16	9	-	-	1	-	-	0.520	1.461
22	Castle Archdale	54.484	7.722	27	4.909	-	0.677	0.688	0.016^{NS}	29	28	1	-	-	-	-	-	0.069	0.414
23	Marble Arch	54.264	7.812	27	5.001	2	0.644	0.708	0.092**	24	21	3	-	-	-	-	-	0.228	0.891
24	Correl Glen	54.439	7.885	24	5.017	-	0.575	0.677	0.155***	24	23	1	-	-	-	-	-	0.083	0.500
25	Coolure	53.677	7.368	24	4.320	-	0.576	0.614	0.065*	25	11	14	-	-	-	-	-	0.513	1.000
26	Tarbet	56.203	4.711	23	4.449	-	0.597	0.662	0.100**	25	23	2	-	-	-	-	-	0.133	0.740

Table 2 Analysis of molecular variance (AMOVA).

Markers	Source of variation	Sum of squares	Variance	% variation
Nuclear	Among populations	159.032	0.06493	1.95
	Within populations	3967.582	3.25746	98.05
Chloroplast	Among populations	36.519	0.04834	18.64
	Within populations	136.469	0.21093	81.36

Table S1 Alder chloroplast microsatellite primers. Multiplex 1 – FN687522, AY165747, FJ012046.1 (6-FAM); Multiplex 2 – FJ012046.2, FJ011994, AY165745 (HEX).

Locus	Repeat	Primers	Size (bp)
FN687522	$(T)_9$	AAAAAGTATTTGAGTATCCTATTTTCG	182
		CAAGAGACATAAAAGAAATTGAAACC	
AY165747	$(T)_{11}$	CAAACAAATAATTGTCAGCAACG	92,93,100
		CGTATGAATTAAGAAGAATTCTTTGG	
FJ012046.1	$(T)_9$	CAGAAAGGATGAAGGATAACCGTA	163
		TCGATTCACAACAACTCTTTCA	
FJ012046.2	$(T)_{12}$	ACATATCATCTCTGATACTGTACTAAAACTT	184,185
		CGGGGCATCATCCTTATTTT	
FJ011994	$(G)_9$	AGACATAATTTCTAATTTCTAATTTTCTTGAG	123,124
		ATTGGGATAGATGTAGATGAATAATAC	
AY165745	$(A)_7T(A)_{10}$	TTTTCCTTGCTCGATTTTGAA	156,157,158
		CGCTTTTGTCAATGACTTGG	

* Forward tailed with CACGACGTTGTAAAACGAC; Reverse tailed with GTGTCTT

Table S2. Population-pairwise Φ_{ST} values. Lower diagonal matrix – nuclear; Upper diagonal matrix – chloroplast. Values significantly different from zero are shown in bold. Numbers refer to populations in Table 1.

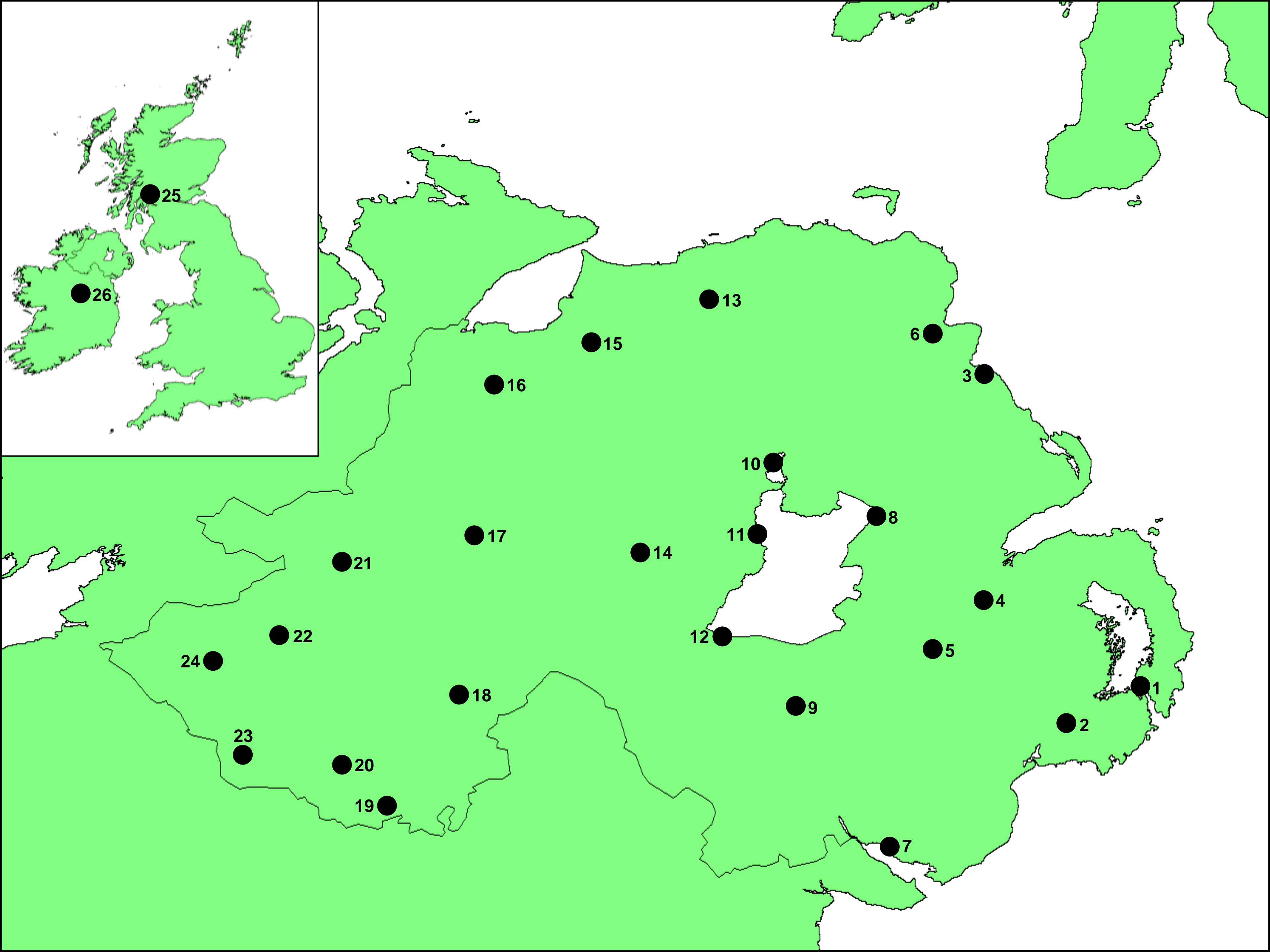
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
1		**0.520**	0.211	**0.290**	**0.610**	**0.319**	**0.696**	0.057	**0.342**	0.042	0.282	0.386	0.229	0.175	**0.394**	**0.286**	**0.553**	**0.560**	**0.308**	0.249	0.385	**0.850**	**0.712**	**0.831**	0.187	**0.776**
2	**0.039**		0.131	0.065	-0.028	0.046	0.003	**0.296**	0.032	**0.339**	0.071	0.006	0.114	0.166	0.005	0.068	-0.056	**0.432**	0.032	0.097	0.000	0.124	0.008	0.100	0.153	0.051
3	**0.048**	**0.019**		-0.027	0.183	-0.014	0.277	0.018	-0.006	0.040	-0.026	0.006	-0.036	-0.034	0.018	-0.027	0.098	**0.406**	-0.009	-0.036	0.021	**0.455**	**0.286**	**0.422**	-0.038	**0.360**
4	**0.050**	0.017	0.005		0.108	-0.036	0.196	0.071	-0.033	0.101	-0.037	-0.031	-0.031	-0.014	-0.022	-0.039	0.031	**0.400**	-0.031	-0.037	-0.019	**0.381**	0.206	**0.346**	-0.021	**0.281**
5	**0.072**	-0.004	-0.013	0.018		0.083	-0.024	**0.363**	0.066	**0.409**	0.113	0.035	0.164	**0.223**	0.031	0.111	-0.058	**0.488**	0.070	0.146	0.026	0.096	-0.021	0.072	0.210	0.019
6	**0.056**	**0.043**	**0.037**	**0.034**	**0.047**		0.164	0.098	-0.034	0.130	-0.032	-0.036	-0.021	0.003	-0.028	-0.034	0.011	**0.403**	-0.031	-0.028	-0.025	**0.338**	0.173	0.306	-0.005	**0.244**
7	**0.053**	0.014	0.008	0.008	0.000	**0.037**		**0.456**	0.144	**0.502**	0.199	0.113	**0.255**	**0.318**	0.102	0.198	-0.016	**0.544**	0.147	**0.238**	0.094	0.032	-0.039	0.015	0.308	-0.021
8	**0.060**	**0.024**	0.004	**0.023**	0.014	**0.048**	0.014		0.115	-0.032	0.069	0.143	0.031	-0.002	0.158	0.070	0.276	**0.447**	0.099	0.041	0.157	**0.616**	**0.465**	**0.587**	0.002	**0.533**
9	**0.049**	**0.023**	0.012	0.011	-0.002	**0.040**	0.007	0.006		0.149	-0.029	-0.040	-0.014	0.014	-0.033	-0.031	-0.003	**0.404**	-0.032	-0.022	-0.031	**0.319**	0.154	**0.288**	0.005	0.224
10	**0.078**	**0.026**	0.011	0.012	**0.028**	**0.052**	**0.023**	**0.037**	0.020		0.098	0.182	0.054	0.015	**0.196**	0.099	**0.327**	**0.481**	0.133	0.067	0.196	**0.659**	**0.512**	**0.633**	0.021	**0.579**
11	**0.055**	0.014	0.002	0.009	-0.034	**0.034**	0.000	-0.005	0.007	**0.018**		-0.027	-0.030	-0.013	-0.017	-0.036	0.037	**0.401**	-0.028	-0.035	-0.014	**0.374**	0.208	0.342	-0.020	**0.280**
12	**0.048**	0.014	0.004	0.014	0.013	**0.036**	0.008	0.016	-0.005	0.014	-0.002		-0.005	0.030	-0.044	-0.029	-0.029	**0.405**	-0.036	-0.015	-0.042	**0.308**	0.124	0.272	0.019	0.200
13	**0.071**	0.016	0.013	0.017	0.002	**0.025**	0.010	0.015	0.010	0.022	0.008	0.010		-0.030	0.007	-0.031	0.081	**0.404**	-0.015	-0.037	0.010	**0.432**	0.264	**0.400**	-0.035	**0.338**
14	**0.065**	0.015	0.016	**0.029**	0.006	**0.059**	0.010	0.008	**0.022**	**0.020**	0.004	0.015	0.024		0.043	-0.013	0.135	**0.414**	0.009	-0.028	0.046	**0.495**	**0.327**	**0.462**	-0.039	**0.401**
15	**0.045**	**0.028**	0.005	0.014	**0.031**	**0.049**	0.005	0.012	0.018	**0.022**	0.007	0.009	0.016	0.018		-0.019	-0.030	**0.412**	-0.028	-0.004	-0.037	**0.277**	0.112	**0.246**	0.033	0.181
16	**0.059**	0.013	0.003	0.009	0.004	**0.040**	0.011	0.011	0.010	**0.017**	-0.004	0.003	0.006	**0.016**	0.013		0.034	**0.400**	-0.029	-0.036	-0.016	**0.377**	0.207	**0.344**	-0.020	**0.281**
17	**0.070**	**0.027**	-0.006	0.007	**0.044**	0.026	0.012	0.008	0.004	0.024	0.000	0.013	0.003	0.017	-0.002	0.011		**0.435**	0.001	0.063	-0.032	0.182	-0.008	0.143	0.122	0.058
18	**0.044**	0.020	0.018	**0.025**	**0.047**	**0.028**	0.010	**0.036**	0.015	**0.043**	**0.020**	**0.024**	0.023	**0.050**	**0.034**	**0.032**	**0.041**		**0.346**	**0.401**	**0.392**	**0.654**	**0.545**	**0.628**	**0.410**	**0.590**
19	**0.056**	0.011	-0.002	0.016	0.014	**0.049**	0.015	0.002	0.014	**0.023**	-0.007	0.009	0.016	**0.017**	0.016	-0.003	0.011	**0.035**		-0.023	-0.029	**0.315**	0.154	0.282	0.000	**0.222**
20	**0.068**	**0.016**	0.009	0.005	-0.012	**0.055**	0.003	0.006	0.013	**0.016**	-0/002	-0.002	0.015	0.006	0.004	0.008	0.001	**0.027**	-0.001		-0.001	**0.423**	**0.248**	**0.389**	-0.033	**0.324**
21	**0.056**	**0.026**	0.015	0.012	0.016	**0.060**	0.019	0.015	0.012	**0.030**	0.014	0.012	**0.028**	**0.023**	0.014	**0.022**	0.006	**0.035**	**0.013**	0.006		**0.263**	0.103	0.231	0.035	**0.169**
22	**0.050**	0.009	0.002	0.011	-0.017	**0.044**	0.001	-0.004	0.012	**0.018**	0.001	0.011	0.010	0.002	0.012	0.009	0.001	**0.023**	0.000	0.005	0.013		0.020	-0.039	**0.493**	-0.018
23	**0.065**	**0.028**	0.015	**0.024**	**0.027**	**0.042**	**0.015**	**0.022**	**0.027**	**0.031**	0.003	0.026	**0.022**	**0.033**	**0.022**	**0.021**	**0.033**	**0.028**	**0.020**	0.021	**0.038**	0.007		0.003	**0.318**	-0.031
24	**0.063**	0.002	0.001	0.018	**0.035**	**0.044**	-0.009	**0.018**	0.001	**0.027**	-0.018	0.018	0.000	0.010	**0.022**	0.000	**0.036**	**0.028**	0.013	-0.001	**0.031**	-0.009	0.014		**0.458**	-0.029
25	**0.061**	**0.030**	**0.026**	**0.038**	**0.034**	**0.036**	**0.039**	**0.044**	**0.036**	**0.052**	**0.026**	**0.030**	**0.016**	**0.057**	**0.041**	**0.027**	0.029	**0.047**	**0.027**	**0.034**	**0.041**	**0.037**	**0.062**	**0.059**		**0.394**
26	**0.064**	0.013	0.005	0.014	-0.003	**0.034**	0.014	0.011	0.012	**0.035**	0.002	0.018	0.010	**0.028**	**0.032**	0.011	0.007	**0.021**	0.000	0.009	**0.022**	0.001	**0.023**	0.008	0.030	

Figure Legends

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

Fig. 2. Distribution of alder chloroplast haplotypes H1-H7. Inset shows the relationships between the seven haplotypes. The dashed line indicates an alternative homoplasious link between haplotypes H4 and H5.

Figs. S1-S11 Bubble plots showing allele frequencies at each locus. Size of bubbles are proportional to allele frequency.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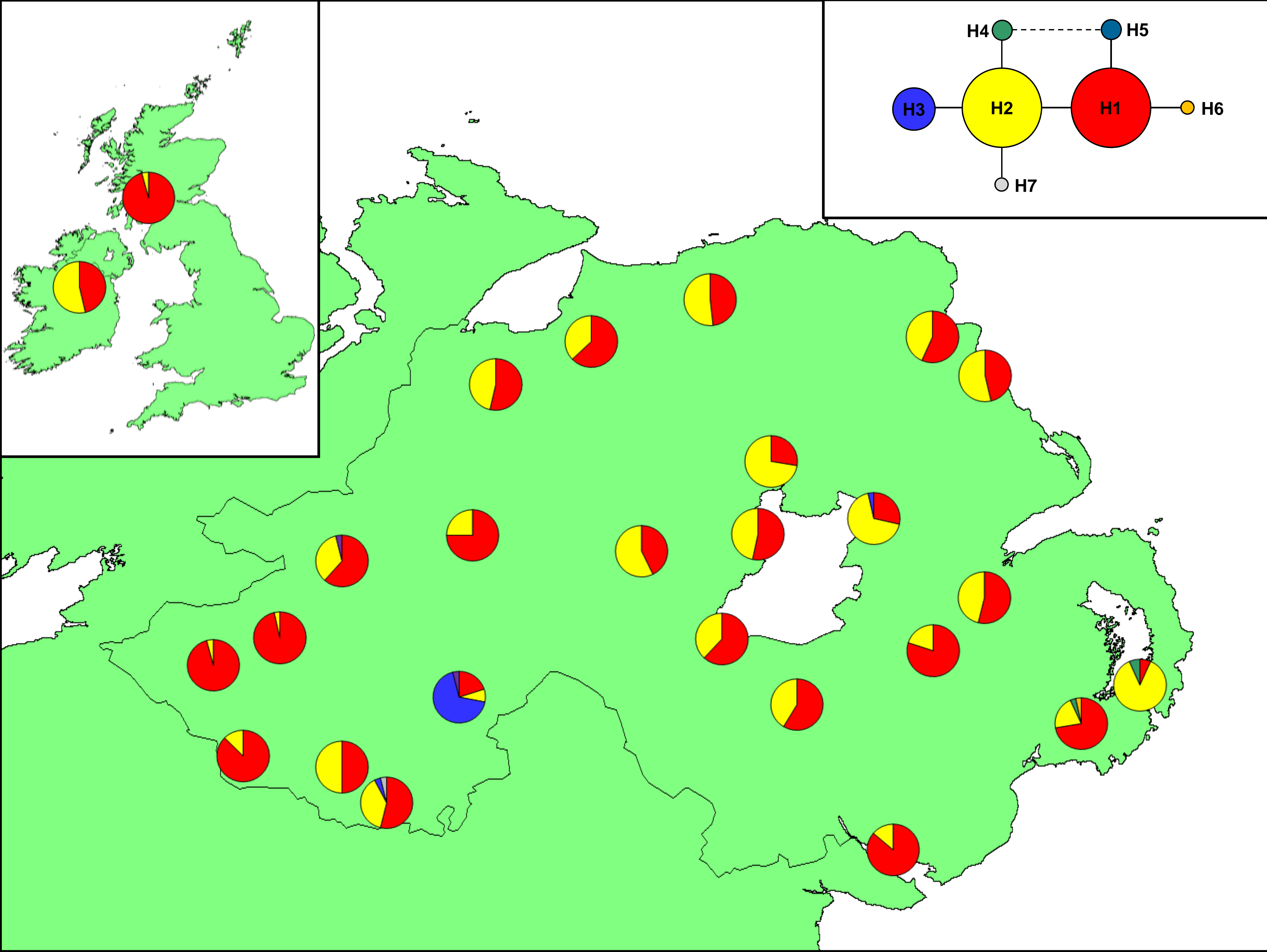
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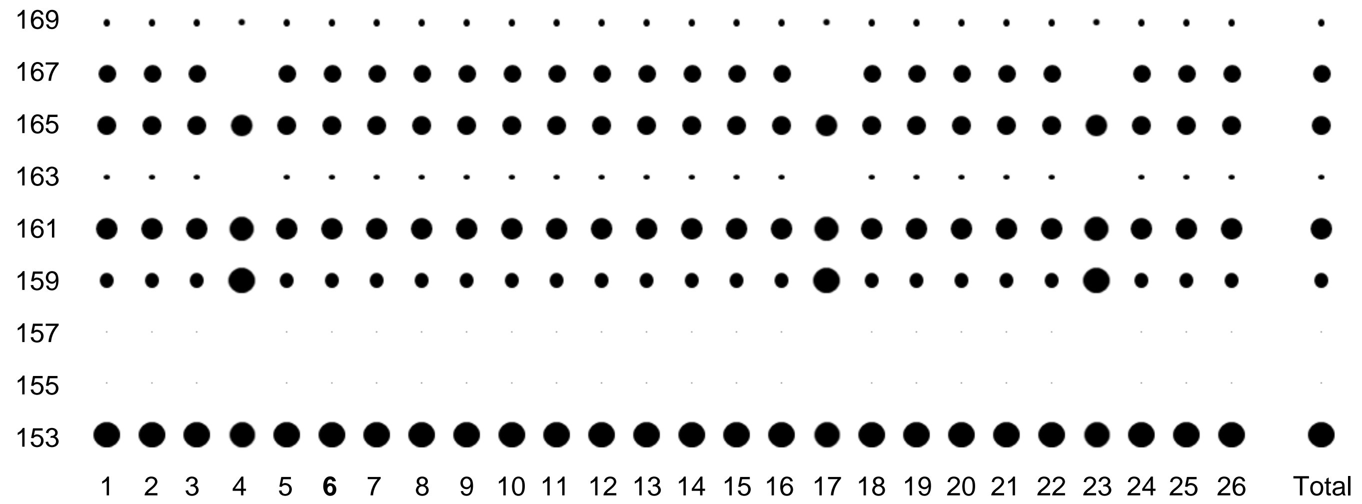
H5
H3
H2
H1
H6
H7



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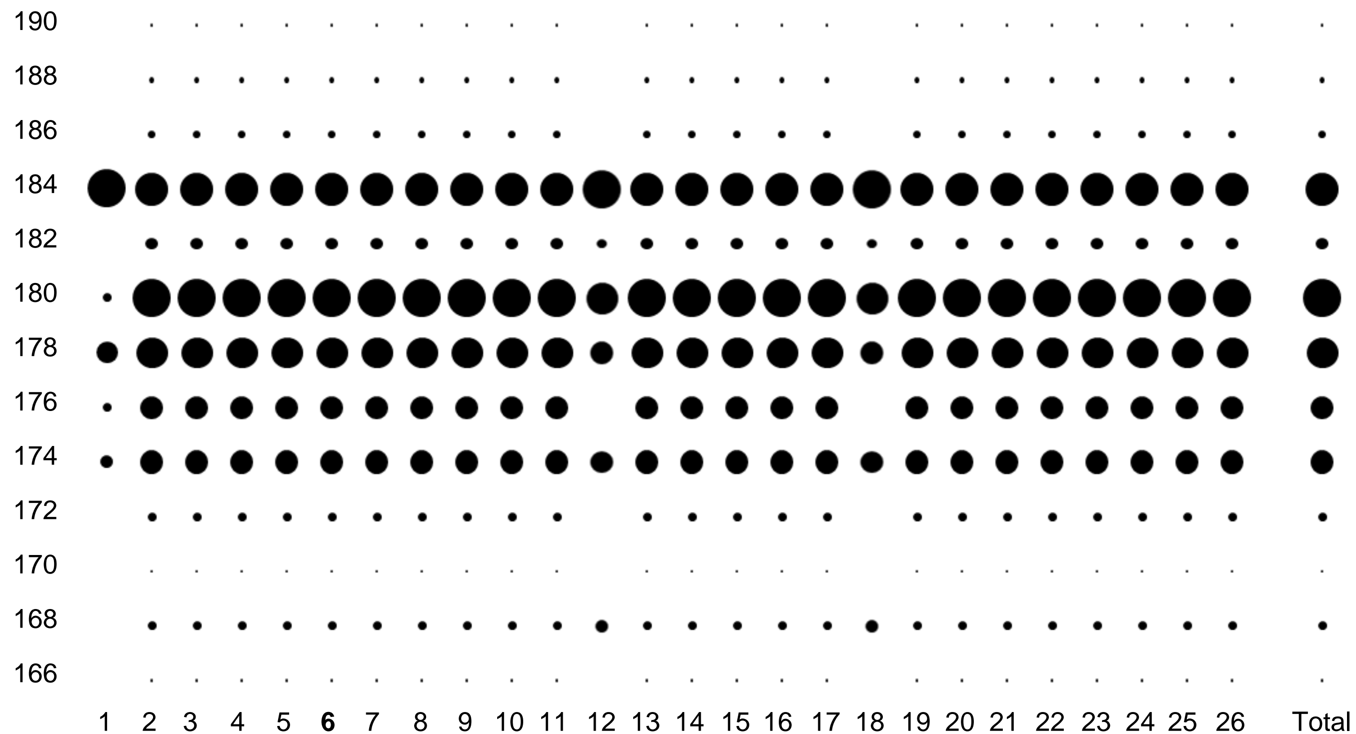

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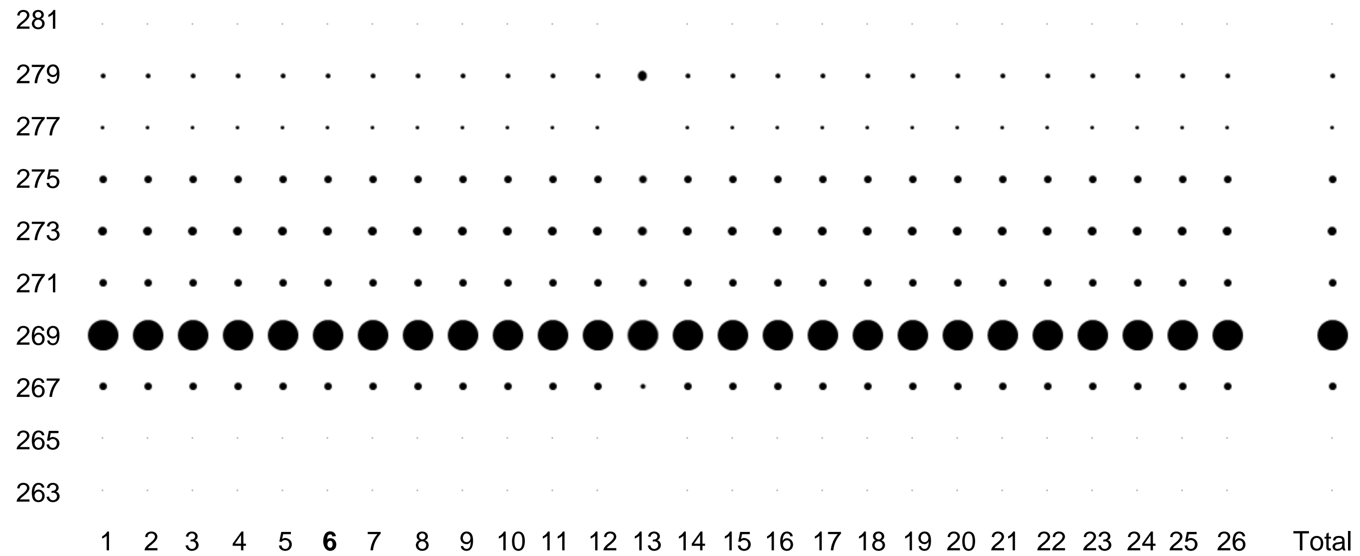

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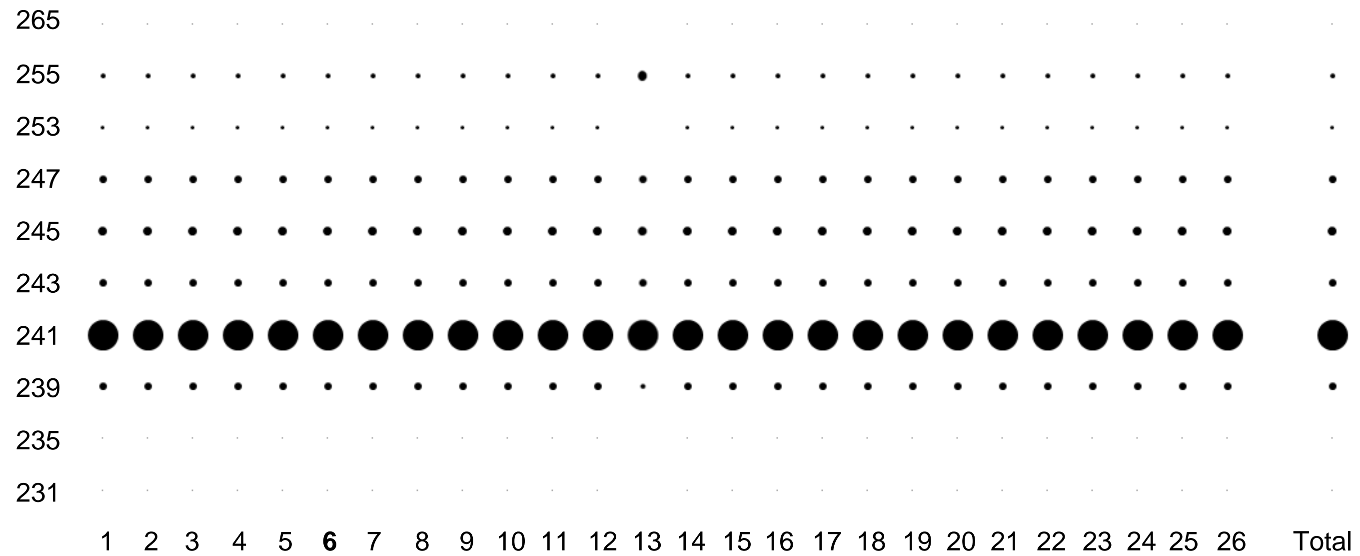

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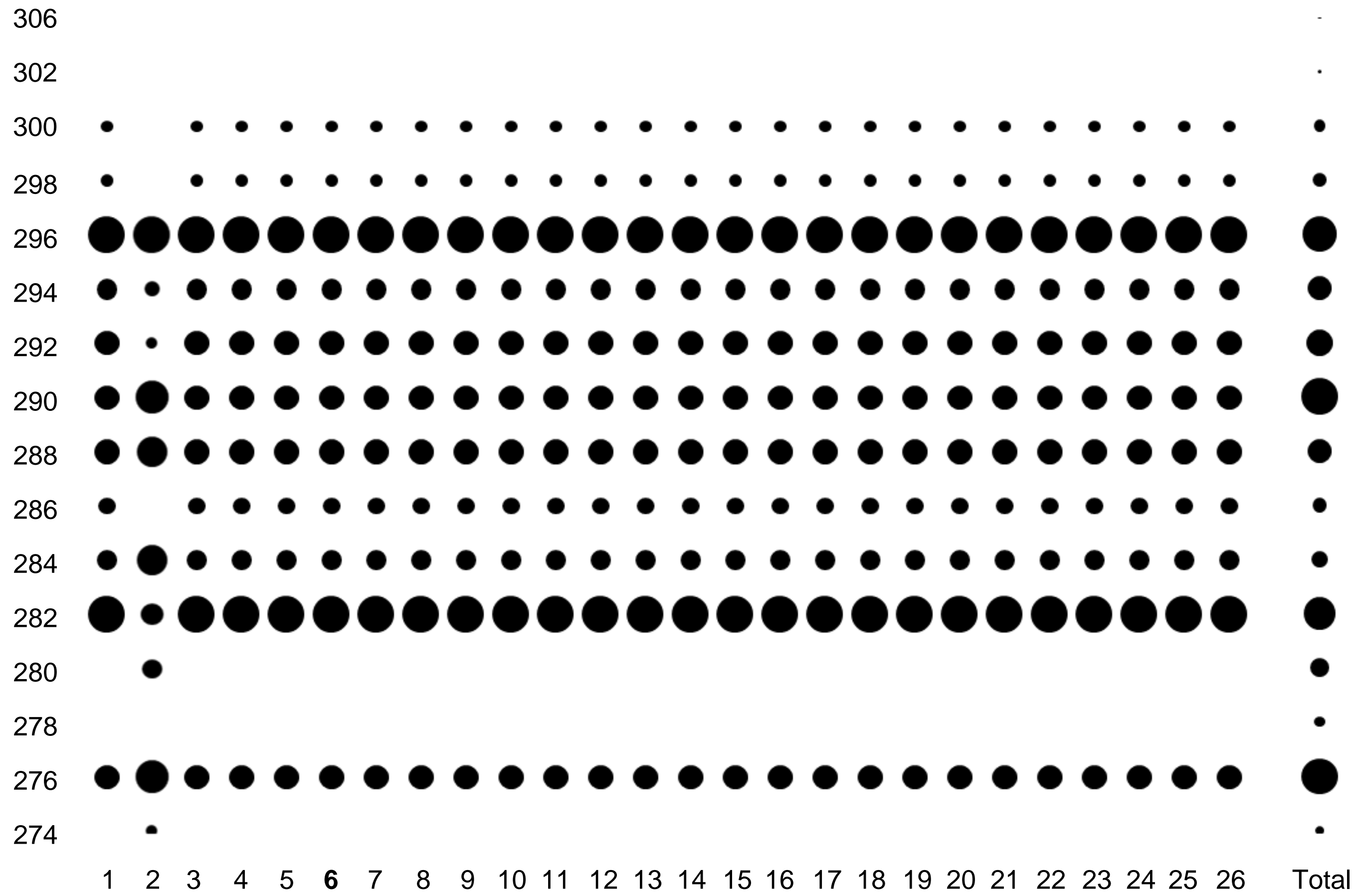

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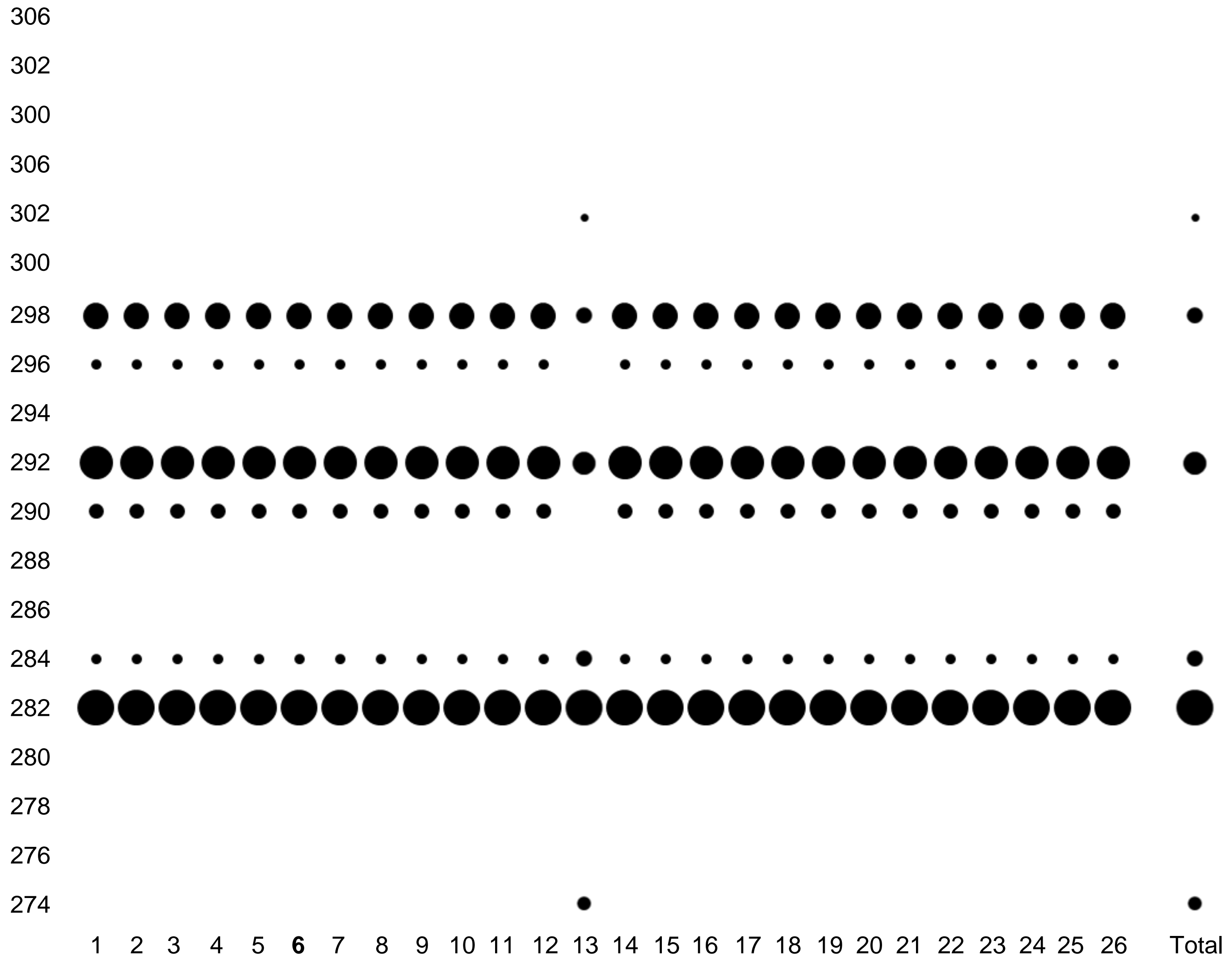

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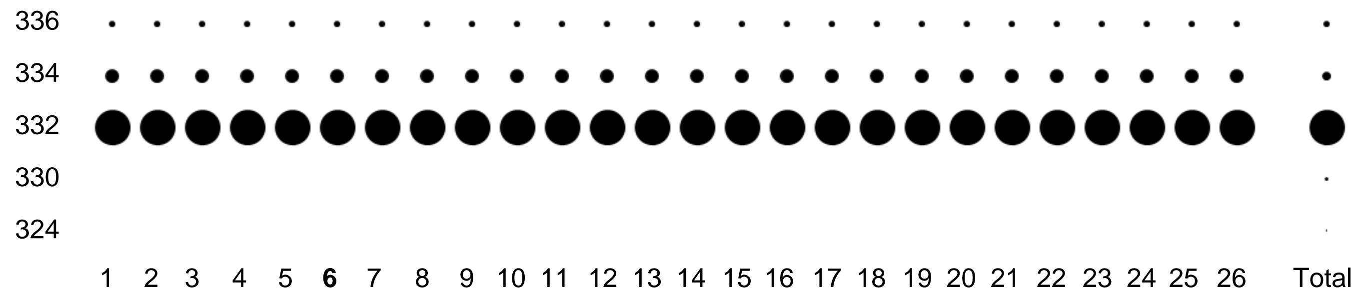

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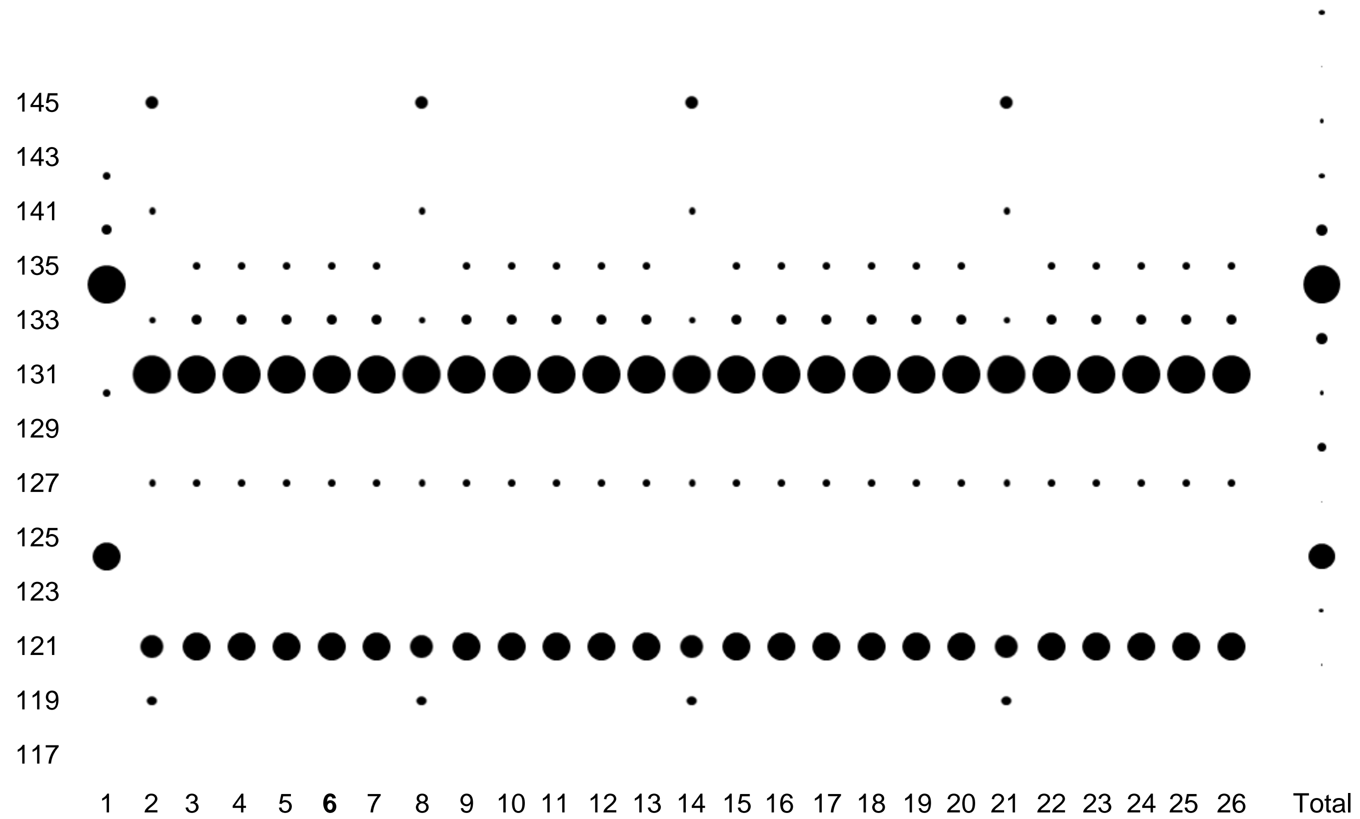

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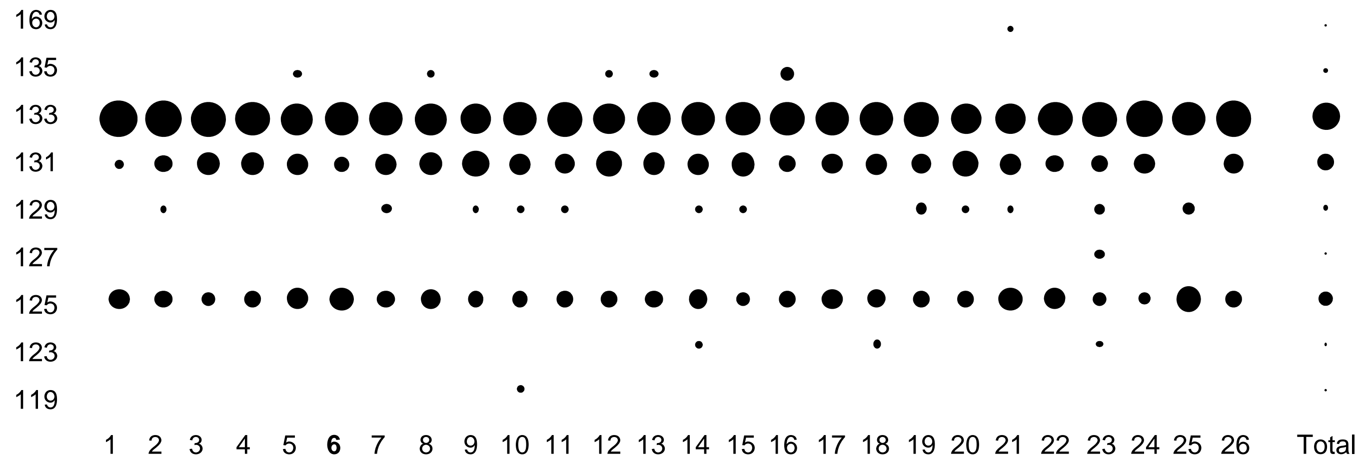

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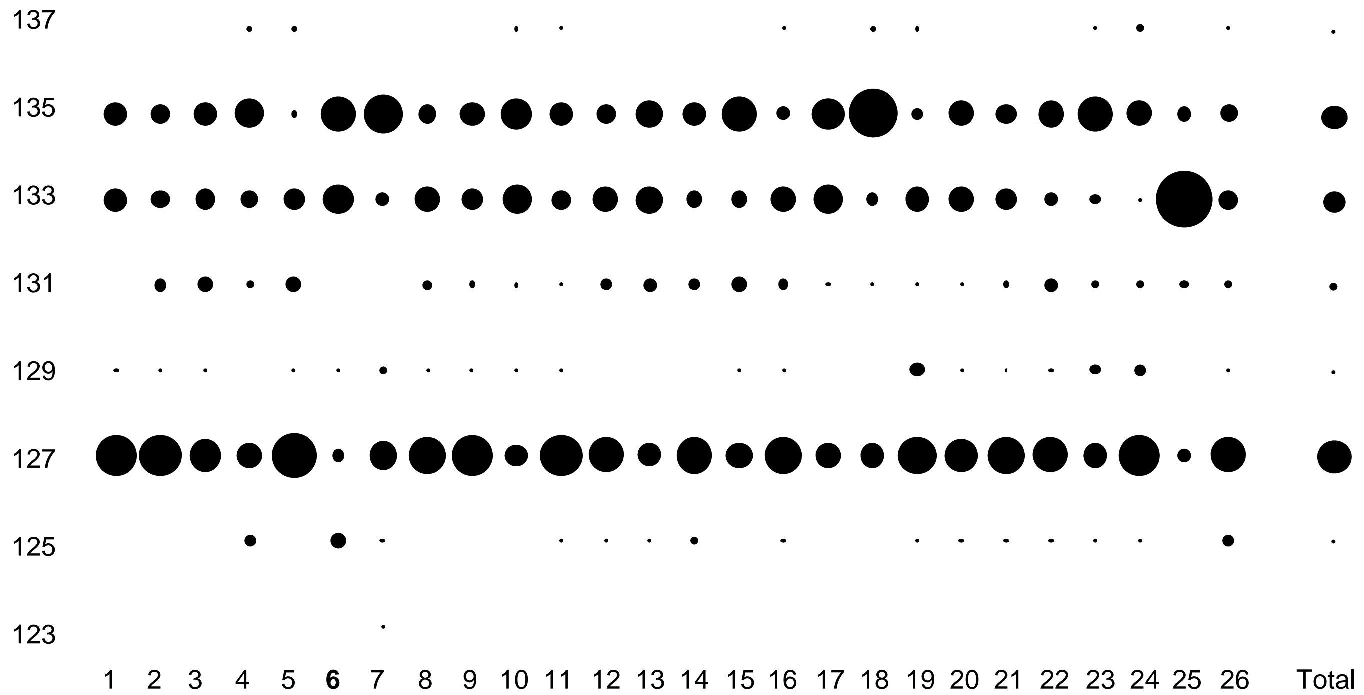

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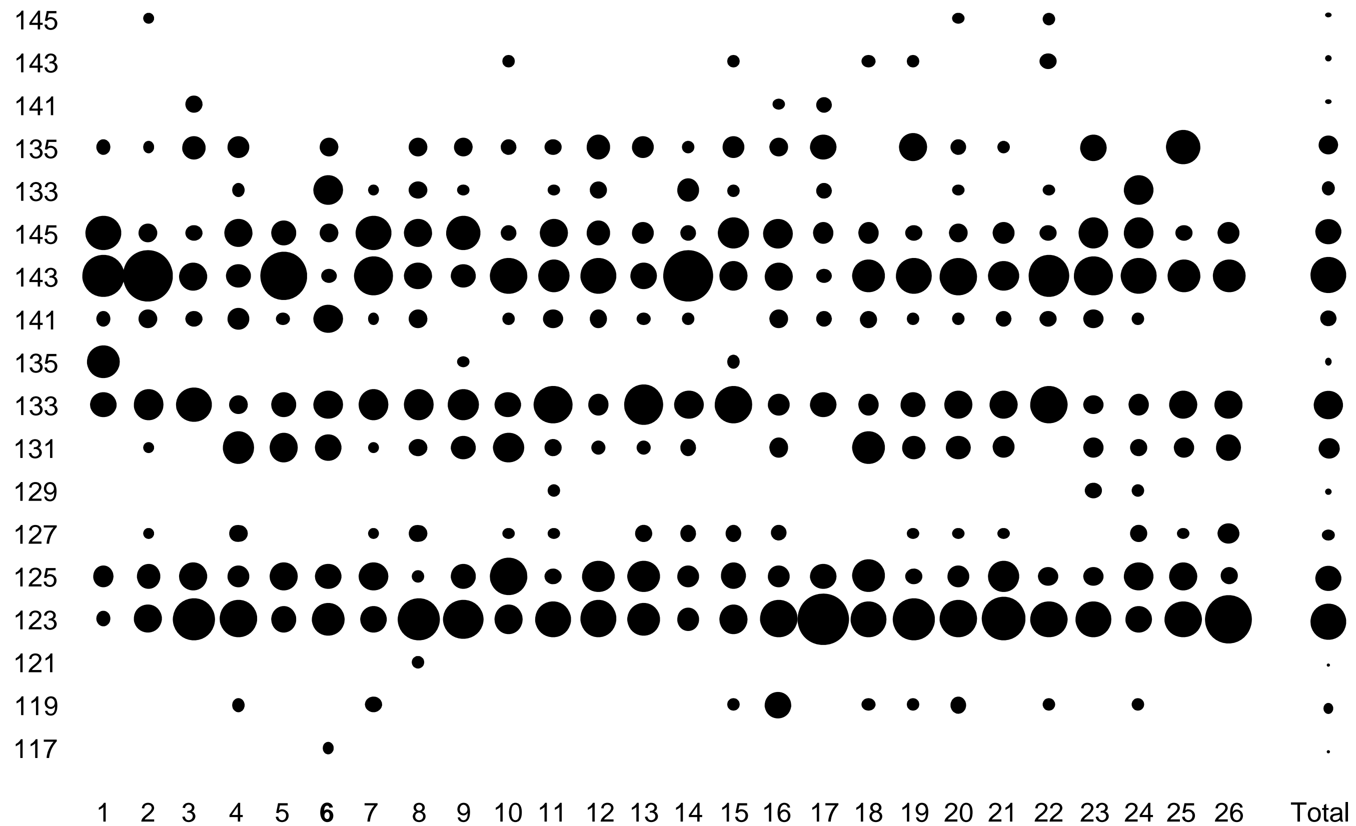

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