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ORIGINAL PAPER

# **Genetic analyses reveal high levels of seed and pollen flow in hawthorn (*Crataegus monogyna* Jacq.), a key component of hedgerows**

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1 **Abstract** Hedgerows represent important components of agri-environment landscapes that  
2 are increasingly coming under threat from climate change, emergent diseases, invasive  
3 species and land-use change. Given that population genetic data can be used to inform best-  
4 practice management strategies for woodland and hedgerow tree species, we carried out a  
5 study on hawthorn (*Crataegus monogyna* Jacq.), a key component of hedgerows, on a  
6 regional basis using a combination of nuclear and chloroplast microsatellite markers. We  
7 found that levels of genetic diversity were high and comparable to, or slightly higher than,  
8 other tree species from the same region. Levels of population differentiation for both sets of  
9 markers, however, were extremely low, suggesting extensive gene flow via both seed and  
10 pollen. These findings suggest that a holistic approach to woodland management, one which  
11 does not necessarily rely on the concept of “seed zones” previously suggested, but which also  
12 takes into account populations with high and/or rare chloroplast (i.e. seed-specific) genetic  
13 variation, might be the best approach to restocking and replanting.

14

15 **KEYWORDS:** *Crataegus monogyna*, gene flow, genetic diversity, hawthorn, microsatellites,  
16 woodland management

## 17 **Introduction**

18

19 Hedgerows represent an important component of agri-environment landscapes, not just as  
20 boundaries for fields, but also in providing a wide range of ecosystem services such as  
21 habitats, food sources and wildlife corridors for animals, acting as wind-breaks, and  
22 preventing soil erosion (reviewed in Burel and Baudry 1995). The last few decades have  
23 seen the emergence of an increasing number of threats to tree and shrub species, including  
24 those which make up hedgerows. These threats include plant pathogens and pests, habitat  
25 loss, land use change, invasive species and climate change (Rackham 2008). Consequently,  
26 managing hedgerow species, especially trees, is becoming increasingly important, requiring  
27 clear knowledge and understanding of their ecological attributes and requirements. Trees lost  
28 due to disease or other factors may have to be replaced under management programmes. In  
29 Great Britain, the Forestry Commission has drawn up a map of 24 areas (seed zones) with the  
30 goal of maintaining provenance by only restocking woodlands with seed from the same zone  
31 (Herbert et al. 1999). These zones are defined by similar geographic, climatic and ecological  
32 characteristics, but it is becoming clear that population genetic structuring can also play an  
33 important role in woodland management (Müller-Starck et al. 1992; Ennos et al. 1998).  
34 Recent studies on ash (*Fraxinus excelsior* L.; Sutherland et al. 2010; Beatty et al. 2015a) and  
35 alder (*Alnus glutinosa* [L.] Gaertn.; Beatty et al. 2015b) found very little genetic  
36 differentiation between woodlands across Great Britain and Ireland, suggesting that the  
37 sourcing of local seeds for replanting might not be required.

38 Natural recolonization, regeneration and succession in plants, including trees, relies on  
39 seed dispersal, which ultimately determines adult vegetation composition (Howe and  
40 Smallwood 1982; Nathan and Muller-Landau 2000; Levine and Murrell 2003). Likewise,  
41 pollen dispersal also has an important role to play in the same processes, especially across

42 deforested landscapes (reviewed in Bacles and Ennos 2008, but see Provan et al. 2008).  
43 Measuring seed dispersal is difficult, with both “tagging” and “trapping” methods having  
44 drawbacks (reviewed in Beatty et al. 2015a). The use of high-resolution chloroplast  
45 microsatellites markers, however, permits the elucidation of maternal gene flow (i.e. via seed  
46 in angiosperms; Provan et al. 2001), and in combination with data from nuclear markers can  
47 indicate the relative importance of seed and pollen flow in natural populations (Ennos 1994).  
48 Furthermore, fine-scale studies wherein both parent plants and saplings are sampled allow  
49 individual parental assignment and the ability to directly measure seed dispersal distances, as  
50 demonstrated in a recent study on European ash (Beatty et al. 2015a).

51 Hawthorn (*Crataegus monogyna*, also known as common or one-seeded hawthorn) is a  
52 species which grows in both woodland and hedgerows, generally occurring primarily in the  
53 latter (Gosler 1990). It prefers areas with a high light intensity and exposed soil, and is adept  
54 at colonising abandoned or eroded areas (Fineschi et al. 2005). Hawthorn hedges provide  
55 habitats for many vertebrate and invertebrate species, and are important nesting sites for  
56 birds, with their thorns providing protection from predators (Pollard et al 1974; Fineschi et al.  
57 2005). The species is indigenous to Europe, and is also found from North Africa to the  
58 Himalayas (Christensen 1992). Like all hawthorns, *C. monogyna* facilitates more than one  
59 group of pollinators, including primarily bumblebees, honey bees and hoverflies (Gyan and  
60 Woodell 1987; Gosler 1990; Campbell et al. 1991). The fruits it produces persist throughout  
61 the winter, acting as an important food source for birds, which are the major vectors for seed  
62 dispersal. These small, fleshy fruits each contain a single seed, and are produced from open  
63 pollination, with self-pollination generally prevented by gametophytic self-incompatibility  
64 (Clapham et al. 1990). However, a study on a British population showed the production of  
65 fruits in the absence of insect pollination, indicating either selfing or apomixis (reviewed in  
66 Jacobs et al. 2009).

67 In recent years, hawthorns have increasingly come under threat from several diseases,  
68 including fire blight, which is caused by the bacterial pathogen *Erwinia amylovora* (Burrill)  
69 Winslow *et al.* (Schroth *et al.* 1974), and leaf spot, which results from a fungal infection by  
70 *Entomosporium maculatum* Lev. (Stowell and Backus 1966). Given that very little is known  
71 on the genetic structure of natural hawthorn populations, and that population genetic studies  
72 on hedgerow species are rare compared to those on woodland trees, we analysed the genetic  
73 diversity in populations of hawthorn on a regional basis across Northern Ireland to inform  
74 management strategies. A combination of nuclear and chloroplast microsatellites was used to  
75 determine the relative importance of pollen- and seed-mediated gene flow, and to facilitate  
76 comparison with woodland species across the same region (Beatty *et al.* 2015a, 2015b, 2016).

77 **Materials and methods**

78

79 *Sampling and DNA extraction*

80 Samples were collected from 23 sites across Northern Ireland along with one site in the  
81 Republic of Ireland (Figure 1; Table 1). Samples were taken from a combination of  
82 hedgerows and woodland, depending on the site. Woodlands were selected that had been  
83 previously designated as ancient or semi-natural based on data collected for the Woodland  
84 Trust Inventory of ancient and long-established woodland in Northern Ireland  
85 ([www.backonthemap.org.uk](http://www.backonthemap.org.uk)) and the National Survey of Native Woodlands 2003–08 in the  
86 Republic of Ireland ([www.npws.ie](http://www.npws.ie)). Woodlands were also selected based on government  
87 information from the Department of the Environment such ASSIs (Areas of Special Scientific  
88 Interest), as well as the landscape character areas listing the woodlands and species present in  
89 each region (<https://www.doeni.gov.uk>). During sampling, 3-4 leaves were taken from each  
90 of a maximum of 30 trees and stored in silica gel. The GPS coordinates of each tree were  
91 recorded. DNA was extracted using the cetyltrimethylammonium bromide (CTAB) method  
92 (Doyle and Doyle 1987). Nuclear genotypes were obtained for between 19 and 30  
93 individuals per population (Table 1;  $N = 677$ ; mean = 28.208), and chloroplast haplotypes  
94 were obtained for between 20 and 30 individuals per population (Table 1;  $N = 701$ ; mean =  
95 29.208).

96

97 *Genotyping*

98 All samples were genotyped for eight nuclear microsatellites (Table 2) and six chloroplast  
99 microsatellites (Table 3). Seven of the nuclear microsatellite loci (excluding CH02D11) had  
100 previously been used in a population genetic study of two different *Crataegus* species, *C.*  
101 *douglasii* and *C. suksdorfii* (Lo et al. 2009). All eight microsatellite markers were originally

102 developed for studies of apple (*Malus x domestica*; Liebhard et al. 2002). To develop *de novo*  
103 chloroplast microsatellite markers, *Crataegus monogyna* chloroplast sequences in the  
104 GenBank database were searched for mononucleotide repeats of nine or more (Provan et al.  
105 2001). Primers were designed using the Primer3 program to amplify the six loci in two  
106 multiplexes, which were combined for a single genotyping run (Table 3).

107 PCR was carried out in a total volume of 10 µl containing 100 ng genomic DNA, 5 pmol  
108 of 6-FAM labelled M13 primer, 0.05 pmol of each M13-tailed forward primer, 5 pmol each  
109 reverse primer, 1× PCR reaction buffer, 200 µM each dNTP, 2.5 mM MgCl<sub>2</sub> and 0.25 U  
110 GoTaq Flexi DNA polymerase (Promega, Sunnyvale, CA, USA). PCR was carried out on a  
111 number of machines: the MWG Primus thermal cycler (Ebersberg, Germany), MJ Research  
112 PTC-200 and PTC-220 Gradient Peltier thermal cyclers (Quebec, Canada) and Biometra T-  
113 Gradient thermal cycler (Göttingen, Germany) using the following conditions: initial  
114 denaturation at 94 °C for 3 min followed by a range of cycles – between 30 and 55 for the  
115 nuclear loci (see Table 2) and 30 for the chloroplast loci – of denaturation at 94 °C for 30 s,  
116 annealing at 58 °C for 30 s, extension at 72 °C for 30 s and a final extension at 72 °C for 5  
117 min. Genotyping was carried out on an AB3730xl capillary genotyping system. (Applied  
118 Biosystems, Foster City, CA, USA). Allele sizes were scored using the GENEMARKER  
119 software (V1.8, Softgenetics).

120

#### 121 *Data analysis*

122 GENEPOP (V3.4; Raymond and Rousset, 1995) was used to test for linkage disequilibrium  
123 between nuclear microsatellite loci. To estimate genetic diversity within the populations,  
124 levels of observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosity were calculated using the  
125 ARLEQUIN software package (V3.5.1.2; Excoffier and Lischer, 2010), whilst levels of allelic  
126 richness ( $A_R$ ) and fixation indices ( $F_{IS}$ ) were calculated using the FSTAT software package



127 (V2.9.3.2; Goudet, 2001). Significance of  $F_{IS}$  was determined by 10,000 randomisation  
128 steps. Chloroplast microsatellite allele sizes were combined into haplotypes, and levels of  
129 genetic diversity ( $H$ ) based on haplotype frequencies were calculated using ARLEQUIN.

130 The overall level of genetic differentiation between populations was estimated using  $\Phi_{ST}$ ,  
131 which gives an analogue of  $F_{ST}$  (Weir and Cockerham, 1984) calculated within the analysis  
132 of molecular variance (AMOVA) framework (Excoffier et al. 1992) using ARLEQUIN. To  
133 further identify possible patterns of genetic structuring, the software package BAPS (V6;  
134 Corander et al. 2003) was used to identify clusters of genetically similar populations using a  
135 Bayesian approach. Ten replicates were run for all possible values of the maximum number  
136 of clusters ( $K$ ) up to  $K = 24$ , the number of populations sampled, with a burn-in period of  
137 10,000 iterations followed by 100,000 iterations. Multiple independent runs always gave the  
138 same outcome.

139 It has been shown previously that the chloroplast genome is maternally inherited in all  
140 Rosaceae tested to date (Fineschi 2005). Thus, the pollen to seed migration ratio ( $r$ ) was  
141 calculated using the formula:

$$142 \quad r = \frac{m_p}{m_s} = \frac{\left(\frac{1}{\Phi_{STn}} - 1\right)(1 + F_{IS}) - 2\left(\frac{1}{\Phi_{STc}} - 1\right)}{\left(\frac{1}{\Phi_{STc}} - 1\right)} \quad (\text{Ennos 1994})$$

143 where  $m_p$  and  $m_s$  are the pollen and seed migration rates respectively,  $F_{IS}$  is the mean  
144 inbreeding coefficient over all loci, and  $\Phi_{STn}$  and  $\Phi_{STc}$  are the levels of genetic differentiation  
145 calculated for nuclear and chloroplast markers respectively.

146 **Results**

147

148 No significant evidence of consistent linkage disequilibrium (i.e. involving the same loci)  
149 was detected between any of the eight nuclear microsatellites analysed (41 out of 694 tests).  
150 Between twelve (CH05G11) and 36 (CH01F02) alleles were detected per locus, with a total  
151 of 206 (mean = 25.75 per locus; Table 2). Levels of observed ( $H_O$ ) and expected ( $H_E$ )  
152 heterozygosity ranged from 0.600 (CH06G11) to 0.924 (CH01F02; mean = 0.764), and from  
153 0.568 (CH05G11) to 0.931 (CH05D04; mean = 0.803), respectively. Levels of  $F_{IS}$  ranged  
154 from -0.033 (CH05G11) to 0.130 (CH03C02), with a mean value of 0.047.

155 Within populations, levels of allelic richness ( $A_R$ ) averaged over loci ranged from 8.779  
156 (Glenarm) to 12.074 (Portaferry) with a mean value of 10.556 (Table 1). Levels of observed  
157 ( $H_O$ ) and expected ( $H_E$ ) heterozygosity ranged from 0.684 (Belleek) to 0.836 (Keady; mean =  
158 0.763), and from 0.769 (Belleek) to 0.838 (Portaferry; mean = 0.803) respectively. Levels of  
159  $F_{IS}$  ranged from -0.022 (Keady) to 0.122 (Belleek) with 13 out of the 24 values being  
160 significantly different from zero. No significant difference was observed for any of the above  
161 diversity measures between hedgerow and woodland populations.

162 Five of the six chloroplast microsatellite loci studied were polymorphic in the samples  
163 analysed, exhibiting between two and seven alleles (Table 3). Combining allele sizes across  
164 loci gave 23 haplotypes, twelve of which were found in a single individual (Table 1). The  
165 most common (H1) was found in 75% (523 out of 701) of the trees studied, and the four most  
166 common haplotypes (H1-H4) accounted for 93% of individuals. Levels of haplotype  
167 diversity ( $H$ ) ranged from 0.193 (Drumshanbo Lough and Belleek) to 0.713 (Eglinton; Table  
168 1).

169 Levels of population differentiation based on the nuclear and chloroplast markers  
170 calculated from the AMOVA were  $\Phi_{ST(n)} = 0.0092$  and  $\Phi_{ST(c)} = 0.0385$  respectively. The

171 BAPS analysis assigned all 24 populations to a single genetic cluster, including the Coolure  
172 population from the Republic of Ireland. Finally, the ratio of pollen to seed migration ( $r$ ) was  
173 2.515.

174 **Discussion**

175

176 The results of the present study, the first to apply high-resolution microsatellite markers to  
177 examine patterns of genetic diversity in *Crataegus monogyna*, indicate high levels of  
178 diversity, but low levels of population differentiation, suggesting extensive gene flow via  
179 both seed and pollen. Previous studies on hawthorn have either used low-resolution  
180 (chloroplast restriction fragment length polymorphism – cpRFLP) markers over large  
181 geographic scales to study the phylogeography of the species, thus focusing more on long-  
182 term historical factors (Fineschi et al. 2005), or have used nuclear markers exhibiting  
183 relatively limited variation (randomly amplified polymorphic DNA – RAPD) at more local  
184 scales (Ferrazzini et al. 2008). The combination of nuclear and chloroplast microsatellites  
185 allows the identification of any fine-scale structuring of genetic variation, as well as  
186 providing insights into the relative contributions of seed and pollen flow in natural  
187 populations (Powell et al. 1996; Provan et al. 2001).

188 The lack of comparable studies (i.e. using similar markers over similar geographical  
189 scales) means that it is somewhat difficult to put the levels of diversity observed in the  
190 present study into context. The sole study examining nuclear genetic variation in *C.*  
191 *monogyna* used dominant RAPD markers (Ferrazzini et al. 2008), and thus it is not surprising  
192 that the reported value of expected heterozygosity (mean  $H_E = 0.291$ ) is much lower than that  
193 in this study (mean  $H_E = 0.803$ ). Likewise, the only other application of nuclear  
194 microsatellite markers to *Crataegus* species was carried out in the polyploid species *C.*  
195 *suksdorfii* and *C. douglasii*, meaning that comparable diversity statistics (e.g.  $H_E$ ) could not  
196 be calculated (Lo et al. 2009). With regard to other tree species from the same region  
197 analysed using microsatellites, levels of diversity in hawthorn were comparable to those  
198 reported in ash (mean  $H_E = 0.765$ ; Beatty et al. 2015a), but slightly higher than those reported

199 for alder (mean  $H_E = 0.663$ ; Beatty et al. 2015b), sessile oak (mean  $H_E = 0.720$ ; Beatty et al.  
200 2016) and pedunculate oak (mean  $H_E = 0.714$ ; Beatty et al. 2016). Levels of inbreeding,  
201 measured as  $F_{IS}$ , were similar to those in ash (Beatty et al. 2015a), but lower than those  
202 reported for alder (Beatty 2015b), probably as a result of the more patchy distribution of the  
203 latter in Northern Ireland.

204 The only previous study using chloroplast markers in *C. monogyna* found four haplotypes  
205 across the whole of Europe using cpRFLP (Fineschi et al. 2005). As in the present study, one  
206 of these haplotypes was found in the majority of individuals studied, including those from  
207 Great Britain (no samples from Ireland were analyzed) but it seems likely that this  
208 corresponds to the dominant haplotype H1 found in Ireland in this study. Interestingly, the  
209 same study found no variation at six chloroplast microsatellite loci, in comparison with the 23  
210 haplotypes from the six loci used in the present study. This is most likely due to the fact that  
211 these six loci are “universal”, rather than species-specific, and tend to exhibit limited  
212 polymorphism in general (Provan et al. 2001).

213 The level of population differentiation observed for nuclear markers ( $\Phi_{ST(n)} = 0.0092$ ) is  
214 over an order of magnitude lower than those reported previously for *C. monogyna* and other  
215 *Crataegus* species. Ferrazzini et al. (2008) reported a value of  $\Phi_{ST(n)} = 0.203$  in six  
216 populations of *C. monogyna* from northern Italy, despite the study area being approximately  
217 the same as that in the present study. This discrepancy is almost certainly due to the different  
218 properties of the markers used, since it has been shown previously that measures of  
219 population differentiation based on RAPD phenotypes, as estimated by Ferrazzini et al.  
220 (2008), are generally around one order of magnitude greater than those estimated using multi-  
221 allelic codominant markers (Isabel et al. 1999). Microsatellite analyses of natural populations  
222 of *C. douglasii* and *C. suksdorfii* yielded  $\Phi_{ST(n)}$  values of 0.22 and 0.37 respectively, although  
223 the latter fell to 0.15 when only diploid samples were included, as much of the differentiation

224 was postulated to arise from some degree of reproductive isolation between individuals with  
225 differing ploidy levels (Lo et al. 2009). It was also inferred that gene flow was further  
226 reduced between populations as a result of apomixis.

227 The fourfold higher  $\Phi_{ST}$  value observed for chloroplast markers ( $\Phi_{ST(c)} = 0.0385$ )  
228 compared to that for nuclear markers ( $\Phi_{ST(n)} = 0.0092$ ) is consistent with the fact that  
229 chloroplast markers have a lower effective population size, and are maternally inherited in  
230 angiosperms and thus dispersed via seed. As a result, they generally show higher levels of  
231 genetic structuring (Provan et al. 2001). Nevertheless, both values are very low, indicating  
232 extensive gene flow across the study area via both pollen and seed. The ratio of pollen:seed  
233 gene flow ( $r = 2.515$ ) is among the lowest reported for a range of plant species (Ennos 1994),  
234 indicating efficient seed dispersal. In hawthorn, this is primarily via ingestion by mammals  
235 and birds, and the latter, particularly thrushes (*Turdus* sp.), are most likely responsible for  
236 long-distance dispersal events (García and Chacoff 2007). Extensive pollen flow is also  
237 expected due to the wide foraging ranges of the bees and flies that pollinate the species  
238 (Steffan-Dewenter et al. 2002).

239 The BAPS analysis indicated that all hawthorn populations studied, including that from  
240 Coolure in the Republic of Ireland, belonged to a single genetic cluster. This was also the  
241 case in ash (Beatty et al. 2015a), and similar to that in alder, where 25 of the 26 populations  
242 studied were assigned to the same cluster (Beatty et al. 2015b). Thus, as in the previous  
243 studies, there is no obvious genetic structuring that could be used as the basis for  
244 management units or seed zones. Nevertheless, examination of the chloroplast haplotypes,  
245 which are maternally inherited and thus indicative of seed diversity, can further inform  
246 management decisions with respect to replanting and restocking. Populations like that at  
247 Portaferry, which contains not only seven of the eleven non-unique haplotypes but also four  
248 unique haplotypes, and that at Eglinton, which has the highest overall chloroplast diversity,

249 would appear to represent good sources of genetic variation. In addition, the Glenarm  
250 population is the only one which is not dominated by the most common haplotype (H1), and  
251 thus may merit special consideration. Conversely, the Belleek and Drumshanbo Lough  
252 populations have the least amount of haplotypic diversity, along with significant  $F_{IS}$  values,  
253 and thus may not represent the best options for the acquisition of seeds for restocking. The  
254 lack of differentiation between the Northern Ireland populations and Coolure might indicate  
255 that, on a broader scale, seeds could be sourced from anywhere in Ireland, but further work  
256 on populations from the Republic of Ireland would be needed to confirm this, and to identify  
257 any potential structuring of chloroplast variation. It should be noted that the use of ostensibly  
258 neutral markers, as in the present study, may not reflect local adaptive variation, which would  
259 only be evident from reciprocal transplant or common garden experiments.

260 It is concluded that extensive pollen- and seed-mediated gene flow occurs in the  
261 populations of hawthorn analysed in the present study, giving rise to high levels of genetic  
262 diversity but low levels of genetic differentiation between populations. These results mirror  
263 those from other woodland tree species from the same region. Thus, there may be an  
264 emerging picture that management of these species may be most efficiently carried out at a  
265 regional level, although a more holistic approach might also target rare or high chloroplast  
266 (seed-specific) variation. Such approaches might not be so suitable for other common tree  
267 species such as hazel (*Corylus avellana*), which have very large seeds and thus may have  
268 much more limited dispersal potential. Further information on such species is needed to  
269 complement the present and earlier findings.

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271

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278 **Data archiving statement**

279

280 All data will be deposited in DRYAD on acceptance.

281 **References**

282

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**Table 1** Details of hawthorn (*Crataegus monogyna*) populations studied. Type – H hedgerow, W woodland; Lat/Long given in degrees; *N* – number of individuals analysed; *A<sub>R</sub>* – allelic richness; *H<sub>O</sub>* – observed heterozygosity; *H<sub>E</sub>* – expected heterozygosity; *F<sub>IS</sub>* – inbreeding coefficient (\* *P* < 0.05; \*\* *P* < 0.01; \*\*\* *P* < 0.001; NS non-significant); H1-H11 – frequency of chloroplast haplotypes (Un – unique haplotypes found in a single individual); *H* – gene diversity.

No	Name	Type	Lat (N)	Long (W)	Nuclear					Chloroplast													
					N	<i>A<sub>R</sub></i>	<i>H<sub>O</sub></i>	<i>H<sub>E</sub></i>	<i>F<sub>IS</sub></i>	N	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	Un	H
1	Portaferry	H	54.394	5.527	28	12.074	0.783	0.838	0.067**	30	17	2	2	2	-	1	-	1	1	-	-	4	0.681
2	Glenarm	W	54.951	5.965	25	8.779	0.729	0.772	0.058*	25	10	2	12	-	-	-	1	-	-	-	-	-	0.627
3	Kilkeel	H	54.065	6.087	30	10.231	0.747	0.809	0.077*	30	22	2	1	3	2	-	-	-	-	-	-	-	0.458
4	Rea's Wood	W	54.710	6.234	30	10.231	0.782	0.802	0.026 <sup>NS</sup>	30	21	4	4	1	-	-	-	-	-	-	-	-	0.490
5	Breen Wood	W	55.140	6.240	29	11.056	0.714	0.804	0.114***	30	20	1	1	5	2	-	-	1	-	-	-	-	0.538
6	Loughbrickland	H	54.292	6.322	30	10.188	0.763	0.798	0.044 <sup>NS</sup>	30	25	2	-	-	1	-	1	-	-	1	-	-	0.308
7	Ballyronan	H	54.701	6.553	27	10.288	0.778	0.793	0.020 <sup>NS</sup>	30	26	-	2	1	1	-	-	-	-	-	-	-	0.251
8	Kilrea	H	54.943	6.573	30	10.273	0.795	0.801	0.008 <sup>NS</sup>	30	25	3	2	-	-	-	-	-	-	-	-	-	0.301
9	Portrush	H	55.195	6.577	30	10.356	0.731	0.795	0.082**	30	21	4	2	3	-	-	-	-	-	-	-	-	0.494
10	Peatlands Park	W	54.486	6.617	29	11.798	0.799	0.812	0.016 <sup>NS</sup>	30	20	2	5	1	-	-	-	-	-	-	-	2	0.538
11	Aghadowey	H	55.015	6.645	30	10.637	0.789	0.812	0.029 <sup>NS</sup>	29	23	1	2	2	-	-	-	-	-	-	1	-	0.372
12	Errigal Glen	W	54.975	6.729	30	11.317	0.775	0.821	0.057*	30	25	3	-	1	1	-	-	-	-	-	-	-	0.303
13	Keady	H	54.282	6.735	30	10.860	0.836	0.818	-0.022 <sup>NS</sup>	30	22	2	2	2	1	-	-	-	-	-	1	-	0.462
14	Killylea	H	54.373	6.841	22	11.001	0.815	0.824	0.011 <sup>NS</sup>	30	24	2	1	1	-	1	-	-	-	1	-	-	0.363
15	Bannagher Glen	W	54.885	6.957	30	9.393	0.739	0.782	0.056*	30	26	-	-	-	-	-	3	-	-	-	-	-	0.246
16	Eglinton	H	55.047	7.214	28	11.612	0.753	0.834	0.099***	30	16	2	2	2	-	-	-	1	-	-	-	4	0.713
17	Plumbridge	H	54.743	7.227	27	10.464	0.806	0.808	0.003 <sup>NS</sup>	28	18	4	1	5	-	-	-	-	-	-	-	-	0.553
18	Lisnaskea	H	54.240	7.395	19	9.649	0.768	0.817	0.061 <sup>NS</sup>	20	14	2	1	-	3	3	-	-	-	-	-	-	0.500
19	Crom	W	54.167	7.454	30	10.271	0.748	0.787	0.050*	30	24	2	2	1	-	-	-	-	-	-	-	1	0.361
20	Drumshanbo Lough	H	54.654	7.493	27	10.580	0.709	0.775	0.087**	30	27	1	-	1	-	-	-	-	1	-	-	-	0.193
21	Lough Erne	H	54.454	7.714	27	10.259	0.723	0.781	0.076**	30	23	3	-	2	1	1	-	-	-	-	-	1	0.409
22	Marble Arch	W	54.266	7.814	30	10.834	0.744	0.808	0.080**	30	26	1	-	1	2	2	-	-	-	-	-	-	0.251
23	Belleek	H	54.465	8.107	29	10.008	0.684	0.769	0.122***	30	28	1	1	-	-	-	-	-	-	-	-	-	0.193
24	Coolure	W	53.671	7.360	30	11.185	0.789	0.814	0.031 <sup>NS</sup>	29	21	3	-	5	-	-	-	-	-	-	-	-	0.451

**Table 2** Hawthorn (*Crataegus monogyna*) nuclear microsatellite loci analyzed in this study. Loci were originally described in Liebhard et al. (2002). Cycles – number of cycles used in PCR;  $N$  – number of alleles;  $A_R$  – mean allelic richness;  $H_O$  – mean observed heterozygosity;  $H_E$  – mean expected heterozygosity;  $F_{IS}$  – mean inbreeding coefficient

Locus	Cycles	$N$	Range (bp)	$A_R$	$H_O$	$H_E$	$F_{IS}$
CH01F02	45	36	213-305	15.600	0.924	0.927	0.004
CH02D11	40	31	159-221	12.067	0.787	0.768	-0.030
CH03C02	40	33	117-193	14.394	0.760	0.871	0.130
CH04G04	40	16	171-213	6.433	0.767	0.768	0.001
CH05D04	40	33	188-258	16.333	0.846	0.931	0.094
CH05D11	45	19	183-231	7.182	0.654	0.709	0.095
CH05G07	40	26	174-224	12.689	0.771	0.883	0.116
CH05G11	30	12	215-247	4.220	0.600	0.568	-0.033

**Table 3** Hawthorn (*Crataegus monogyna*) chloroplast microsatellite loci analyzed in this study.

Multiplex	Accession	Region	Repeat	Primers*	Alleles (bp)
1	EU500411	<i>rpl2-trnH</i> intergenic	(A) <sub>9</sub>	ATAAAAACAAAATAGGAGTAATTAATTGTGAC TTCTTAATAAATGATTTGCTACAAAAGG	100,101
	JQ390913	<i>rpl20-rps12</i> intergenic	(T) <sub>13</sub>	TATAACCTTCCCGACCACGA ATTTACTACTTTTATGTGTTTTTGATACCT	119,121,122,123
	JQ392044	<i>trnG-trnS</i> intergenic	(A) <sub>9</sub>	GATTCGTTGGAACAATAAATGG GGATTGAAAGAGCCCTTCATAA	135
2	JQ391567	<i>rpl16</i> intron	(T) <sub>9</sub>	TTGCTTTACAACCCATAATCAGA ACCAACTCATCACTTCGTGTT	159,161,162,163,164,166
	HG764984	<i>trnH-psbA</i> intergenic	(T) <sub>13</sub> ...(A) <sub>10</sub>	AGATAAAATACAACCTAAATTGAAAATT ATATATGAGTTCTTGAAAGTAAAGGAGTAA	195,196,219,220,221,222,223
	FJ395291	<i>atpF-atpH</i> intergenic	(T) <sub>10</sub>	CATTTTTCATATGATATCCTCTTTCTT CGGGTACCTAATTCTAATAAGTATCATTC	244,246

\* Forward tailed with CACGACGTTGTAACGAC; Reverse tailed with GTGTCTT



**Table 4** Analysis of molecular variance (AMOVA) for nuclear and chloroplast microsatellite markers analysed in hawthorn (*Crataegus monogyna*).

Markers	Source of variation	Sum of squares	Variance	% variation
Nuclear	Among populations	105.747	0.02811	0.92
	Within populations	4006.636	3.01251	99.08
Chloroplast	Among populations	10.378	0.00833	3.85
	Within populations	140.752	0.2079	96.15

## Figure Legend

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

The dashed box in the top-right map show the area in Europe which the study was carried out.

